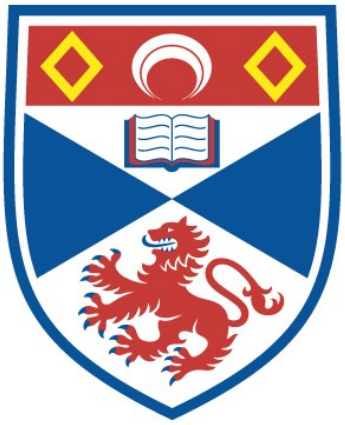
A STUDY ON THE UROMYCES PISI GROUP OF RUST FUNGI

Daniel Wilson Burns

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



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A STUDY ON THE *UROMYCES PISI* GROUP OF RUST FUNGI

by

DANIEL WILSON BURNS, B.Sc.

A Thesis submitted to the University of St. Andrews for the Degree of Doctor of Philosophy.

Department of Botany,
University of St. Andrews.

June, 1954.

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CAREER.

I first matriculated in the University of St. Andrews in October 1947 and graduated with B.Sc., with Honours of the Second Class in June 1950.

In October 1950, I was admitted as a Research Student in the University of St. Andrews under Ordinances 16 and 61 and was awarded a Maintenance Grant from the Department of Scientific and Industrial Research. From July 1951 to September 1952, I was appointed a Research Assistant in the Department of Plant Pathology, Cornell University, Ithaca, N.Y., U.S.A. On my return to this country I was again admitted as a Research Student in the University of St. Andrews under the above Ordinances and was awarded a Maintenance Grant from the Department of Scientific and Industrial Research. During session 1953-54 I was awarded a University of St. Andrews Post-graduate Scholarship.

In April 1954, I was appointed Lecturer in Botany in the University of Aberdeen, which appointment I now hold.

DECLARATION.

I hereby declare that the following Thesis is based on the record of work done by me, that the Thesis is my own composition, and that it has not previously been presented for a Higher Degree.

The research was carried out in the Department of Botany at the United College of the University of St. Andrews under the direction of Dr. J. A. Macdonald.

CERTIFICATE.

I certify that Daniel Wilson Burns has spent nine terms of Research Work under my direction and that he has fulfilled the conditions of Ordinance No. 16 (St. Andrews), and that he is qualified to submit the accompanying Thesis in application for the degree of Doctor of Philosophy.

ACKNOWLEDGMENTS.

I wish to record my deep appreciation to Dr. J. A. Macdonald of the Department of Botany, United College, for supervising the work presented in this Thesis and for the continued interest he has shown in the problem.

I am also indebted to Mr. J. Gray, M.A., of the Department of Mathematics, United College, for his advice on statistical methods employed in this work, and to Professor Allen, of the Department of Natural Philosophy, United College, for the use of special equipment, and to Mr. H. Cairns of the same Department for constructing the Voltage Doubler Unit of the cytophotometer.

I would also like to thank Dr. A. H. Jarret of the Department of Astronomy, United College, for the many discussions I had with him on optical problems in microscopy.

THE
UROMYCES PISI GROUP
OF
RUST FUNGI.

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I. INTRODUCTION.

The work which is presented in this thesis, was started in 1949. At that time the investigations were confined to a study of the life cycle of *Uromyces punctatus Schroet.*, since this species had been reported as a new record for Britain, (Macdonald, 1949). The results of thi study were presented in the form of a thesis for the honour degree in Botany in 1950. The main point brought out in that work was, that although *U. punctatus* was a Hetero-Uromyces species on the continent, at St. Andrews it appeared to be a Hemi-Uromyces species. In view of this, it was decided to continue investigations on this rust and extend them as a post-graduate research topic, to include the near relations of *U. punctatus*.

The group, referred to hereafter as the *Uromyces pis* group, includes the following seven species of rust fungi:- *U. punctatus Schroet.* *U. pisi (Pers. Ex. D.C.) Wint.*, *U. loti (Kirch.) Blytt*; *U. striatus Schroet.*, *U. laburni (D.C.) Fuck.*, *U. jordianus Bubak*, and *U. fischeri-eduardii Magn.* All these species are found on the continent, thoug only the first four are known for the St. Andrews district. *U. striatus* is rare. With the exception of *U. jordianus* for which no aecidial stage has yet been found, the members

of the group are heteroecious. The teleuto stage is confined to the Leguminosae, in some cases, e.g. U. jordian to a few species of a single genus, and in other cases, e.g. U. pisi, to the species of more than one genus. This plurivorous nature of the teleuto stage, e.g. U. striatus and U. loti have been recorded on the same species of Lotus has led to some confusion in the literature. The aecidial stage, however, occurs only upon a few species of Euphorbia and this fact has caused some difficulties in identificatio The situation is similar to that obtaining in the heteroecious Puccinia graminis Pers.

From the morphological point of view, the aecidial stage presents the greatest problem in separating the species, for in all cases the aecidium appears to be identical. According to Moehrke (1927), and Guyot (1939), there are differences in the symptoms produced on Euphorbia by the various species of the group, but in general it is necessary to inoculate the teleuto stage hosts with aecidiospores, before the species concerned can be determined (Constantineanu (1920). This point will be discussed later.

In the teleuto stage, the morphological characters of the individual species are more distinct. In some case there are only slight differences in such characters as the

number of germ pores of the uredospores, the sizes and shapes of these spores and the teleutospores, and the sculpturing on the walls of the latter. On the other hand, there is considerable difference between the extremely fine punctations on the teleutospore wall of U. pisi and the comparatively large longitudinal striations of U. striatus. Nevertheless, all sorts of wall markings exist which are intermediate between these types, and there is also some overlapping of the spore measurements.

It was because of these intermediate characters that Jørstad (1948), after studying the literature and carrying out a few inoculation experiments, suggested that the U. pisi group was a heterogeneous species composed of a number of complex races. According to this view, the members of the group, distinguished by other authors as distinct species, are relegated to the rank of variety or race, collectively forming one species.

The questions which naturally arise are:- do the members of this group of rust fungi form one heterogeneous or composite species; or are they individual species which are obviously closely related? It is the aim in this thesis to examine the problem and to provide an answer to these questions.

Before any progress can be made in such an investigation, it is necessary to determine what criteria will be used in delimiting the species. It is proposed, therefore, to review briefly the literature on this point and then to discuss these criteria, and others applied to the U. pisi group by the writer.

II. CRITERIA USED IN DELIMITING SPECIES OF RUST FUNGI.

(a). Literature review.

Over 150 years ago, each form of rust was described according to its general appearance, and to a certain extent, by its parasitic habit. Persoon (1794, 1801), was the first to classify the rusts after the binomial system of Linneaus had been introduced. His generic names were based on the type of sorus, and the parasitism of the species was reflected in the specific epithet. Link (1809), appears to consider the parasitic nature of the rusts as being fundamentally important, for he separates them from other fungi because they grow on living plants, although later (1815), he removed Gymnosporangium from this group, - his endophytae - and placed it in association with Tremella. As the microscope was improved, it became possible to describe and feature these microfungi in greater detail. For example Leveille (1847), separated the rusts into three classes, (i) those with paraphyses - his 'cystidia', (ii) those without paraphyses, and (iii) doubtful Uredineae. He also made use of the pedicellate or sessile character of the teleutospores and whether or not they were catenulate. Up to this time then, the main criteria of a species were its gross morphology and its

parasitic habit. It was believed at that time that each spore type was a separate organism, and that where both uredo- and teleutospores occurred in the same sorus, one was parasitic upon the other.

What may be considered to be the modern period, was ushered in with the publication in 1853 of De Bary's, 'Die Brandpilze'. He had studied a great number of rusts using new culture methods, and showed that a species of rust was composed of a sequential development of spore types. The classification he devised at that time, emphasised the importance of the pycnidia. A year later (1854), Tulasne, proved that the uredospores and teleutospores were not independent organisms, nor were they parasitic upon one another. This dimorphism was embodied in his classification, where the teleutospores were regarde as 'true spores', and the uredospores and aecidiospores, as 'stylospores'. It was Tulasne then, who first placed real emphasis on the teleutospore. In 1865, De Bary demonstrated for the first time the heteroecious nature of certain rust fungi, and pointed out their narrow specialisation in the selection of hosts.

Such discoveries as polymorphism and heteroecism were slow to be accepted by other mycologists. The idea that a rust fungus could change from one form to another of

unlike form, accompanied, at least in certain species, with a complete change of host, was so contrary to the beliefs then held that it was in some measure opposed, and it is no surprising that it was not until about 25 years later that the importance of these discoveries was realised. For example, Schroeter in 1870, and later in 1889, introduced a classification which took account of the life cycles. This made use of prefixes which described the number of spore types present. His first paper dealt with the genus Uromyces and in the later one he extended the scheme to other genera. Winter (1884) to a certain extent, Plowright (1889), Hariot (1892), and Bubak (1908), all followed the Schroeterian scheme of separating the species first by life cycle, then by morphology and host reaction. Winter (loc. cit.) keyed out his genera by the teleutospore character, and the species by their life cycle.

The pioneering work of Eriksson (1894), Eriksson & Henning (1894), and Hitchcock & Carleton (1894), emphasised once more the delicate specialisation of the rust fungi. A number of floras and monographs published after this, all reflected the importance of this phenomenon; e.g. Sydow (1904-12), Trotter (1908), Hariot (1908), Fischer (19 and Migula (1910), all used the host reaction first and the separated the species by morphology, usually that of the

teleutospore. Fischer (loc. cit.) also makes use of the heteroecious or autoecious nature of the species.

In 1889, the rusts were assigned to the Basidiomycete by Brefeld, and four years later Van Tieghem endorsed this view. Vuillemin (1893) had pointed out the significance of the basidium and this influenced Van Tieghem's classificatio of 1898, when he separated the rusts into two families depending on whether the basidium was internal or external to the teleutospore.

Dietel in 1897, and later in 1900, published his classification of the rusts and this has been the basis of all subsequent work. The order was divided into four families, and the chief diagnostic feature was the morphology of the teleutospore. Later, in 1928, he reduce the number of families to two. Thus, the importance of the teleutospore was again underlined. Dietel's classificatio is reflected in that of Grove (1913), where the families are distinguished according to whether the teleutospore is pedicellate or impedicellate, and the genera are separated by the morphology of the teleutospore. The species are characterised by their host reaction.

Arthur (1906, 1907-27, 1934) attempted a classificati combining morphological and biological characters and takin into account the full life cycle of any species. Although

his contribution to mycology and especially to our knowledge of rust fungi has been of inestimable value, his classification required much more knowledge of the group than was available at that time, and has led to much unnecessary confusion.

The work of the cytologists at the turn of the century endorsed the views of the rust taxonomists in considering the teleutospore to be the most important spore Sappin-Trouffy (1896), and Holden & Harper (1903), for instance, demonstrated first the dikaryotic nature of the mycelium and the eventual fusion of the two nuclei in the teleutospore.

In the above rather brief review of the more important classifications it is brought out that there are three features which have been applied with varying emphasis in the characterisation of a species of rust. These are, broadly speaking, morphological considerations, specialised parasitism, and the length of the life cycle. It is also indicated that as the knowledge of the group increased so did the number of factors used in separating species.

(b). <u>Criteria used in the present study</u>.

Modern taxonomy, although chiefly concerned with the grouping of organisms into systems for convenience of

identification, also seeks the natural causes of likenesses and differences in character observed, and it does this knowing that true relationship has as its basis, a communit of origin. (Sharp, 1943). Greater use has been made of the diversity of evidence, and to a large extent descriptio has been supplemented by experiment. Apart from morphological data, use is also made of physiological behaviour, ecological relations and geographical distribution, cytological characters, genetical behaviour in controlled crosses, and to a certain extent serological reactions and the fossil record. In the present study it was impossible to apply genetical methods because the sexual stage of the fungi being studied was absent at St. Andrews. Serological methods and the fossil record deal more with phylogenetical relationships than with specific ones, and consequently were also neglected.

Before going on to describe the application of the other types of evidence to the U. pisi group, it is desirable to emphasise the peculiar difficulties which are likely to be encountered.

In the Uredinales we have not only to deal with the diversity, but also the intergradations of morphological forms, in many cases reminding one of the perplexities met in Rubus, Rosa, Viola, and other phanerogamic groups.

In addition we have to deal with the physiological requirements of strict parasitism, embracing a delicate specialisation, and also heteroecism with its unrelated hosts (Arthur, 1929).

(i) Morphological data.

These features usually include colour, size and shape of the various parts, and their arrangement. In the higher plants, on account of their macroscopic size, it is comparatively simple to describe these characters. There is no difficulty, for instance, in counting the numbers of sepals, petals, and stamens, and their arrangement is usually quite obvious. The rusts are microfungi, and because of this, recourse must be had to the microscope to determine the sizes, shapes and configurations etc., of the various spores. There must also be a greater precision in making spore measurements.

Unfortunately, there has been no consistent use of a satisfactory method of describing spores in the rust literature, and often the terms used are vague. Hariot (1892), for example, in his paper on the leguminous rusts, drew attention to the poor descriptions of the teleutospore. "Les caractères tirés de la surface lisse ou verruqueuse de la spore sont rarement indiqués avec exactitude par les descripteurs....." To take a definite example, Grove

(1913, p. 94), describes the teleutospores of U. pisi as "subglobose to ovate, with a small hyaline papilla (as much as 3 μ high), everywhere minutely and rather densely verruculose, brown, 20-28 x 14-22 μ; epispore 1.5 μ thick; pedicels hyaline, short, deciduous." and Plowright (1889, p. 134) describing the same spores of the same fungus says, "subglobose or shortly elliptical, finely but closely punctate when recent, apex only slightly thickened, 20-30 x 17-20 μ. Pedicels long, colourless, fragile." The vagueness of the method is brought out in a comparison of the two descriptions. For instance, the sizes of the spores in both cases are of the same magnitude, but there is no indication as to whether the mean lies at one end of the range or the other. Whether the pedicels are long or short in fact is also a difficult point to decide. Then again, whether "everywhere minutely and rather densely verruculose", means the same as, "finely but closely punctate when recent", is a moot point. Admittedly, the description of the wall of the spore is not easy, but there seems to be no reason why descriptions of this nature should not be substantiated by photographs or drawings, after the style of Migula (1910).

Consequently, in this study where the above features, i.e. the size, and sculpturing of the walls of the spores,

and spore wall thickness, are used to characterise the species, the measurements were treated statistically and the sculpturing of the walls was photographed by the phase contrast microscope. The actual methods used will be fully discussed later in the appropriate section. As regards the uredospores, the wall markings were neglected as there appeared little or no difference between the members of the U. pisi group, but the number and distribution of the germ pores were considered.

(ii) Physiological data.

In general, physiological behaviour may be taken to represent the response of plants to their supply of nutritive substances. Applied to the rusts, it may be interpreted as parasitic specialisation, for the fungus is obviously nourished by its host, although the exact nature of this process is not known.

As mentioned earlier, Eriksson (1894), and others, demonstrated that specialised varieties can exist within one species of rust. The general conclusion from this, is that each variety must obtain a special chemical substance essential to its existence from its special host. The only way in which these special chemical substances can be differentiated at present, is by host reaction. Most

of the work on specialisation has been carried out with cereal rusts, and it has been emphasised that the relationship existing between the fungus and its host is essentially dynamic. Two other important findings have emerged from these studies: (1) A host plant may inherit resistance to rust infection according to Mendelian laws. This has led to extensive breeding programmes with economic crops susceptible to rust infection. (2) The discovery of heterothallism and the subsequent hybridisation studies on races etc., have suggested that in nature crossing may result between races or physiologic forms with the possible production of new races or forms, and perhaps, eventually, species. Further, these new races or forms may change their selection of special host. Apart from this, there is natural adaptation to contend with. One of the great difficulties in these studies has been that the genotypic character for rust resistance is rarely expressed phenotypically and must be measured by pathogenicity tests.

In view of this specialisation of rust fungi, it is clearly not practical to describe species without regard to their special host plants. It is necessary, therefore in describing the results of infection experiments to give, as far as possible, a history of the hosts used - their

specific or varietal name, and whether cultivated or wild, - and also that of the rust, with indications as to the purity of the cultures.

In the experiments which are described later, the conditions of temperature, humidity etc., of the incubation chamber were controlled as far as possible, and the source and history of the host plants and rusts used are given.

(iii) Ecological and Geographical data.

This is one of the features of the rusts about which little is known. Essentially each species is limited in its distribution by that of its host, but the two distributions need not be identical. There can be no doubt that geographical barriers, which are conducive to the formation of new species and races of higher plants, operate to the same extent in the case of rust fungi. It appears from the literature, that it is mainly the life cycle of these fungi which is affected by their geographical distribution, e.g. in Europe, U. acetosae has all spore forms in the south, but towards the north, or on top of southern mountains, it forms only teleutospores (Arthur, 1929). Climatic conditions will of course effect the host plant as well as the rust. The general opinion amongst uredinologists seems to be, that there is a tendency

for the life cycle of rusts to become shortened in northern latitudes.

The bearing of this on the life cycles of the members of the U. pisi group will be discussed later.

(iv) Cytological data.

In recent years cytologists have played a leading role in the delimitation of the species. The cytologist concerns himself chiefly with the number of chromosomes, their size, and general morphology, i.e. the species has a constant and characteristic set of chromosomes. However, as Clausen (1936) points out, there is a limitation to a purely cytological interpretation of a species, for he has shown that identical morphological groups, which were also identical cytologically, may be genetically heterogeneous. Thus the cytological delimitation of a species can only operate when there is a visible morphological difference in the chromosome complement. Anderson (1937) for example, has shown that certain races of Tradescantia which were hardly distinguishable morphologically, could be separated after a study of the chromosome morphology, and further, that these cytological races were well defined geographically. One of the fundamental contributions that cytology has made to the species concept, is that it may

indicate not only a difference between species or group of species, but may also demonstrate the way in which these differences came about.

The contribution of cytology to the taxonomy of the rust fungi has not been great. As Ramsbottom (1949), points out, "... the number, size, and shape of the chromosomes must not be over-valued taxonomically, for in the fungi the nuclei are so small." Nevertheless, there have been a few claims as to the chromosome number in certain rusts, e.g. Holden & Harper (1903) described 6 to 10 chromosomes for Coleosporium solidaginis, while Olive (1942, 1949) and Sanwal (1953) mention 8 for the same genus. Recently, M'Ginnis (1954) has described a chromosome number of 6 for Puccinia coronata. It is well known, however, that the nuclei of the genus Coleospori are comparatively large, reaching a diameter of 13 u in the fusion nucleus (Olive 1949). In the U. pisi group the nuclei are only 3-4 μ in diameter and the chromosomes can only be seen with great difficulty (Plate VI, figs. 32, 33).

Apart from having a constant chromosome complement, it has been shown in recent years that somatic nuclei contain a fixed amount of desoxy-ribose nucleic acid (D.N.A. per nucleus, and this amount is characteristic for each species (Vendrely & Vendrely, 1948, 1949, Davidson &

McIndoe, 1949, Thomson, 1953). It has also been shown that a haploid nucleus has half the amount of D.N.A. of a diploid nucleus of the same species. These facts have been demonstrated both by biochemical analysis and by light absorption (cytophotometric) techniques (Pollister & Ris, 1947, Swift, 1950, Moses, 1952, and others).

Chemical analysis of nuclei cannot easily be carried out with the rust fungi because of the intimate association of these organisms with their host plants. However the cytophotometric method lends itself well in the case of rusts, because ordinary Feulgen stained preparations of paraffin sections are used in the analysis. This method has been employed in the present study and will be fully described later.

The above criteria are those which have been employed in the present study. The following sections will present the data for each criterion separately.

III. MORPHOLOGICAL DATA.

As mentioned in the introduction, characters to be considered in this section are, for teleutospores, the length of the longest and shortest diameters (referred to hereafter as length and breadth), the thickness and the sculpturing of the wall, and the thickness of the pore cap; for uredospores, the lengths and breadths, the thickness of the wall, and the number and distribution of the germ pores. These are the factors which, after a careful preliminary examination of a large number of specimens, appeared to assist in the characterisation of each species, and had a fair degree of constancy within each species.

(a) Literature review.

It is proposed to review first of all the literature on the spore measurements of the U. pisi group and to discuss this, then present for comparison similar data obtained by the writer from various harbarium and local specimens. The sculpturing and the thickness of the wall, and the number and distribution of the germ pores are treated separately in a similar manner.

(i) Teleutospores.

Table 1 presents a summary of the teleutospore measurements of U. striatus, obtained from the literature.

Tables 1 and 2.

Author	Length	Breadth
Schroeter (1871)	19.9 - 24.6	18 - 21
Saccardo (1888)	18 - 28	14 - 20
Schroeter (1889)	20 - 25	16 - 18
Fischer (1904)	19 - 24	16 - 20
Sydow (1904-12)	18 - 24	15 - 20
Bubak (1908)	17.5 - 26.5	15.5 - 20
Trotter (1908)	18 - 28	14 - 20
Arthur (1912)	19 - 24	15 - 20
Grove (1913)	18 - 24	15 - 20
Migula (1917)	20 - 28.5	10 - 20
Guyot (1938)	18 - 21 (19-20)	16 - 20 (17-18)
" (1946)	20 - 23	16 - 18
" (1951)	18 - 23 (20-22)	14 - 18 (15-17)

Table 1. U. striatus. Measurements of teleutospores in microns.

Author	Length	Breadth
Karsten (1879)	26 - 30	20 - 22
Cooke (1878-9)	26 - 30	20 - 22
Winter (1884)	19 - 32	17 - 20
Saccardo (1888)	20 - 32	17 - 21
Schroeter (1889)	20 - 30	17 - 20
Fischer (1904)	20 - 31	14 - 22
Sydow (1904-12)	20 - 28	14 - 22
Bubak (1908)	22 - 35	15.5- 26
Trotter (1908)	18 - 32	15 - 21
Hariot (1908)	20 - 32	17 - 21
Grove (1913)	20 - 28	14 - 22
Migula (1917)	20 - 31	14 - 22
Guyot (1951)	19 - 27 (20-25)	15 - 21 (15-18)
Rayss (1951)	21 - 27	18 - 21

Table 2. U. pisi. Teleutospore measurements in microns.

The first column gives the author and reference, and the second column, the measurements in microns of the spores given in the work quoted. In some cases, where an auther has published the measurements for a particular species more than once, e.g. Migula (1910 and 1917), and if the measurement are the same, only one reference is quoted in the tables. In other cases, however, where an author publishes more than once and gives different measurements in these works for the same species, e.g. Schroeter (1871, and 1889), both sets of measurements are recorded. Sometimes an author quotes the measurements given by an earlier author, e.g. Plowright (1889), had no acquaintance with U. pisi as a British species, but gives the measurements of Schroeter published earlier that year (1889). Where it is known that this occurs, the later reference - in this case Plowright (1889) - is omitted. It will be convenient when discussing the tables to abbreviate 'the range of the lengths' to range (L), and similarly for breadths, range (B).

Considering table 1, it will be seen that Schroeter has published two different sets of measurements. Comparing the two sets, the measurements of the lengths are roughly in the same range, but those of the breadths have different ranges. There is a difference, too, in the ranges (B) given by Guyot (1946, 1951) and the range (B) given by

Tables 3 and 4.

Author	Length	Breadth
Schroeter (1871)	16 - 18.8	21 - 24
Saccardo (1888)	17 - 22	15 - 16
Schroeter (1889)	17 - 22	15 - 16
Hariot (1892) a.	17 - 22	15 - 16
b.	20 - 24	16 - 18
Fischer (1904)	17 - 25	15 - 21
Jordi (1904)	17 - 23	15 - 21
Sydow (1904-12)	14 - 24	14 - 21
Bubak (1908)	20 - 31	15.5 - 22
Trotter (1908)	17 - 25	15 - 21
Guyot (1951) a.	14 - 28 (17-25)	13 - 22 (15-20)
" " b.	19 - 27 (21-25)	15 - 20 (17-18)
Rayss (1951) a.	20 - 26	17 - 20
" " b.	24 - 26 (20-25)	19 - 21 (17-20)
" " c.	24 - 25	18 - 20

Table 3. U. punctatus. Teleutospore measurements in microns.

Author	Length	Breadth
Fischer (1904)	18 - 23	15 - 21
Jordi (1904)	18 - 23	15 - 21
Sydow (1904-12)	17 - 25	14 - 21
Bubak (1908)	18 - 26	15 - 21
Trotter (1908)	19 - 25	16 - 20
Saccardo (1912)	17 - 25	14 - 21
Guyot (1951) a.	18 - 27 (20-24)	14 - 18 (15-17)
" " b.	20 - 26 (22-24)	15 - 19 (16-18)
Rayss (1951)	17 - 25	14 - 21

Table 4. U. loti. Teleutospore measurements in microns.

Shroeter (1871). The ranges (L) given by Guyot (1938), and Migula, overlap to a certain extent, but if the means (L) are considered to be somewhere in the middle of these ranges, it is seen that there is a difference of about 5 μ between the two means.

Table 2, presents similar data for U. pisi. Although the measurements appear to have a uniformity of range, there are a few exceptions. Compare, for instance, the ranges (L) given by Cooke, and by Karsten, with that of Guyot (1951). Guyot's range, though it overlaps that of the other two, suggests that the mean (L) of the spores is somewhere between 20 and 25 μ, whereas the means of Cooke, and of Karsten, lie between 26 and 30 μ. Again a difference of mean values of about 5 μ. It will be noted, however, that these two ranges occur within the range (L), given by Trotter. In the third column, Schroeter, and Winter, both give a range (B) of 17-20 μ, whereas Cooke, and Karsten, both give 20-22 μ. Here again, both ranges are different and both can be included within that of Bubàk.

In table 3, the data for U. punctatus are given. In this table, the ranges vary more than in tables 1 and 2. For instance, the range (L) of Schroeter (1871), definitely conflicts with the ranges (L) given by Bubàk, Guyot (1951, b. Hariot (1892 b), and Rayss (1951 a, b, c). A similar

Tables 5, 6 and 7.

Author	Length	Breadth
Fischer (1904)	17 - 25	14 - 21
Jordi (1904)	17 - 23	16 - 21
Sydow (1904-12)	15 - 22	14 - 20
Migula (1910)	15 - 22	14 - 20
Saccardo (1912)	15 - 22	14 - 20
Guyot (1951)	15 - 25	14 - 21

Table 5. U. jordianus. Teleutospore measurements in microns.

Author	Length	Breadth
Sydow (1904-12)	24 - 30	19 - 25
Bubak (1908)	22 - 33	22 - 24
Migula (1910)	24 - 30	19 - 25
Saccardo (1912)	24 - 30	19 - 25

Table 6. U. fischeri-eduardii. Teleutospore measurements in microns.

Author	Length	Breadth
Saccardo (1888)	18 - 28	14 - 22
Schroeter (1889)	18 - 22	14 - 16
Sydow (1904-12)	16 - 28	14 - 20
Bubak (1908)	17.5- 33	15.5- 22
Trotter (1908)	18 - 28	14 - 21
Hariot (1908)	18 - 28	14 - 22
Migula (1910)	18 - 22	14 - 16
Wilson (1934)	16 - 28	14 - 20
Guyot (1951)	17 - 27 (20-25)	15 - 20 (16-18)

Table 7. U. laburni. Teleutospore measurements in microns.

difference exists between the ranges (L) given by Rayss (1951, b, c), and those of Schroeter (1889), of Jordi, and of Saccardo, and to a lesser extent, of Sydow. Considering the breadths, Hariot's two ranges (B) (1892 a, b) are at variance with each other, and both lie outside the range (B) given by Schroeter (1871). Further, the ranges (B) of Rayss (1951 a, b, c), do not agree with those of Hariot (1892 of Saccardo, and of Schroeter (1889). The ranges (B) quoted by all the authors in table 3, with the exception of that of Schroeter (1871) are all within the range given by Guyot (1951a). The same is true of the ranges (L), the exception being Bubàk.

The ranges (L and B) given in table 4 for U. loti, have a large measure of agreement and require no comment.

The same remarks apply to the data on U. jordianus and U. fischeri-eduardii given in tables 5 and 6 respectively. There has been little work done on these two species, and no doubt some of the ranges quoted are the original figures, but they are presented here because it was not clear from the literature whether original work was being quoted.

Table 7 deals with U. laburni. Like tables 4-6, the ranges are fairly constant, except that the ranges (B) of Schroeter, and of Migula, tend to lie outside that of Bubàk.

Tables 8 and 9.

Author	Length	Breadth	G.P.
Schroeter (1871)	17-21 diam.	-	-
Saccardo (1888)	17-22 diam.	-	-
Schroeter (1889)	18-22 diam.	-	-
Fischer (1904)	17-21 diam.	-	4 usually
Sydow (1904-12)	15-22 diam.	-	3 - 4
Bubak (1908)	15.5-24	15.5-20	3 - 4
Trotter (1908)	17-23 diam.	-	4
Arthur (1912)	18-23	16-20	3 - 4
Grove (1913)	15-22 diam.	-	4 - 6
Migula (1917)	17-21 diam.	-	-
Guyot (1951)	18-23 (20-22)	16-19 (17-18)	-

Table 8. U. striatus. Uredospore measurements in microns and number of germ pores (G.P.)

Author	Length	Breadth	G.P.
Cooke (1878-9)	20-24 diam.		-
Karsten (1879)	20-24	20-25	-
Winter (1884)	17-24 diam.		-
Saccardo (1888)	17-24 diam.		-
Schroeter (1889)	17-20	25	-
Klebahn (1892)	18-21 diam.		3 - 4
Fischer (1904)	21-25 diam.		4 - 5
Sydow (1904-12)	21-25 diam.		3 - 5
Bubak (1908)	22-39.5	20-24	4 - 5
Trotter (1908)	18-25 diam.		4 - 5
Grove (1913)	21-25 diam.		3 - 5
Migula (1917)	17 - 25	25	4 - 5
Rayss (1951)	21-24	18-21	-

Table 9. U. pisi. Uredospore measurements in microns and number of germ pores (G.P.)

Tables 10 and 11.

Author	Length	Breadth	G.P.
Schroeter (1871)	21-28	19-21	-
Saccardo (1888)	20-24	18-20	-
Schroeter (1889)	20-24	18-20	-
Hariot (1892)	20-24 diam.		-
Fischer (1904)	18-24	16-22	3 - 4
Jordi (1904)	18 - 24	16-22	3 - 4
Sydow (1904-12)	15-24	14-22	3 - 4
Bubak (1908)	20-28.5	17.5-22	3 - 4
Trotter (1908)	18-24	22-26	3 - 4
Rayss (1951) a.	20-23 diam.		3
" " b.	20-28	20-23	3 - 4
" " c.	20-25	18-23	3 - 4

Table 10. U. punctatus. Uredospore measurements in microns and number of germ pores. (G.P.)

Author	Length	Breadth	G.P.
Fischer (1904)	18-25	17-23	2 - 5
Jordi (1904)	18-25	17-23	2 - 5
Sydow (1904-12)	17-25	16-23	2 - 5
Bubak (1908)	18-25	17-23	2 - 5
Trotter (1908)	18-25	17-23	2 - 5
Grove (1913)	17-25	16-23	2 - 5
Saccardo (1912)	17-25	16-23	2 - 5
Arthur (1934)	18-27	16-23	-
Guyot (1951) a.	23-28 (24-26)	19-24 (22-23)	3 - 5
" " b.	21-28 (22-25)	19-23 (20-22)	3 - 4
" " c.	21-28 (22-25)	20-24 (21-22)	3 - 4

Table 11. U. loti. Uredospore measurements in microns and number of germ pores.

(ii) Uredospores.

The corresponding data for the uredospores are given in tables 8-14.

From table 8 (U. striatus), it is obvious that the shape of the spores is almost spherical and that the ranges given are in good agreement.

The ranges given for U. pisi in table 9, are also in good agreement.

The data for U. punctatus are presented in table 10. From the table it is seen that the spores are slightly longer in one diameter compared with those in tables 8 and 9. The majority of the ranges (L) agree well but the breadths show some differences. For instance, Schroeter (1889), and Saccardo, give ranges (B) which are well outside those of Schroeter (1871), Rayss (1951, b), and Trotter. Jordi's range (B) lies outside that of Trotter's.

The ranges quoted for U. loti (table 11), show a fair degree of constancy, which was noted for the teleutospores (table 4). Guyot's ranges (L) (1951 a, b, c), are somewhat higher than those of the other authors.

The data for U. jordianus, U. fischeri-eduardii and U. laburni are given in tables 12, 13 and 14, respectively. These ranges agree well except the breadths in table 14, where the range (B) given by Guyot lies outside that given by Bubàk.

Tables 12-14.

Author	Length	Breadth	G.P.
Fischer (1904)	18-28	17 - 23	6 - 8
Jordi (1904)	18-25	17 - 23	6 - 8
Sydow (1904-12)	17-24 diam.		6 - 8
Bubak (1908)	18-26 diam. or 20-28	17 - 23	6 - 8
Migula (1910)	18-26 diam. or 20-28	17 - 23	6 - 8
Saccardo (1912)	17-24 diam.		6 - 8

Table 12. U. jordianus. Uredospore measurements and number of germ pores. (G.P.)

Author	Length	Breadth	G.P.
Sydow (1904-12)	20-26 diam.	20 - 22	3 - 5
Bubak (1908)	22		4 - 5
Migula (1910)	20-26 diam.		3 - 5
Saccardo (1912)	20-26 diam.		3 - 5

Table 13. U. fischeri-eduardii. Uredospore measurements and number of germ pores. (G.P.)

Auther	Length	Breadth	G.P.
Schroeter (1889)	22-28	18-22	-
Sydow (1904-12)	20-28	18-24	3-6
Bubak (1908)	22-31	20-26.5	3-6
Trotter (1908)	19-32	17-24	-
Migula (1910)	22-28	18-22	3-6
Saccardo (1888)	19-32	17-24	-
Wilson (1934)	20-28	18-24	3-6
Macdonald (1946)	form a. 21.1-28.8 (24.6)	18.6-22.5 (20.7)	-
	form b. 20.9-29.3 (23.9)	18.9-28.1 (22.1)	3-4
	form c. 21.7-38.4 (28.6)	18.3-21.9 (20.3)	-
Guyot (1951)	19-27 (22-25)	16-19 (17-18)	3-5

Table 14. U. laburni. Uredospore measurements and number of germ pores. (G.P.)

The data in tables 1-14, which are discussed above, show that there is considerable variations in the ranges (L and B) given by different workers for the same species. Because of these variations within a species, it is difficult to compare one species with another, for, with the exception of Guyot (1938, 1946 and 1951), no indication is given of the number of spores measured, or whether the spores were mounted in a liquid medium or under dry conditions, and the mean of the measurements is never expressed.

Consequently, in order to obtain a rough comparison of the species, the data in tables 1-14 were used in two ways. Firstly, the lowest and highest measurement of the ranges (L and B) for each species, was taken from the tables, and expressed as the 'extreme range'. For example, in table 14, the lowest measurement of the range (L) is 19 µ and the highest for the same range is 32 µ. Thus the extreme range (L) for this species is considered to be 19-32 µ. Similarly for the ranges (B). Secondly, the mean of the lower and of the higher measurements for each species was expressed as the 'mean range'. For example, in table 14, the mean of the lower measurements of the range (L) is 20.4 µ and that of the higher measurements of the same range is 29.4 µ, and hence the mean range (L) is given as 20.4-29.4 µ. The mean range (B) was determined in a like manner. The data obtained by these

Table 15.

Species	Extreme range		Mean range	
	L	B	L	B
Teleutospores				
U. punctatus	14-31	13-24	18.5-24.2	16.1-19.6
U. loti	17-27	14-21	17.3-24.4	15-20.1
U. striatus	17.5-28.5	14-21	19-24.7	15.1-19.4
U. pisi	18-35	14-26	20.8-31.5	17-21.3
U. jordianus	15-25	14-21	15.6-23.1	14.3-20.5
U. fischeri-eduardii	22-30	19-25	23.2-28.2	19.7-24.7
U. laburni	16-33	14-22	17.9-26.7	14.4-19.3
Uredospores				
U. punctatus	15-28.5	14-28	18.8-24.6 or 20-23.5 diam.	18-22.8
U. loti	17-28	16-24	18.9-26	17.4-23.2
U. striatus	15-24	15.5-20	17.1-23.3 or 16.6-21.7 diam.	15.8-19.6
U. pisi	17-39.5	18-25	19-27 or 19.2-24.5 diam.	19.4-23.7
U. jordianus	17-28	17-23	18.6-27 or 17.5-25 diam.	17-23
U. fischeri-eduardii	20-26 diam.			
U. laburni	19-32	16-26.5	20.4-29.4	17.7-23

Table 15. Summary of spore measurements obtained from the literature. Figures in microns.

two methods are presented in table 15.

Considering first the teleutospores (table 15) it can be seen in the extreme range column, that it is impossible to separate any of the species either by ranges (L) or (B), since all of the ranges overlap. However, in the other column, it is possible to separate U. fischeri-eduardii from U. jordianus by range (L), but not by breadths. The range (L) of the former differs from that of U. punctatus, U. loti, U. striatus and U. laburni, although there is some overlapping in all cases. In the ranges (B), U. fischeri-eduardii could be separated from U. punctatus, U. striatus and U. laburni.

The ranges given in the first column for the Uredospore all intergrade with one another, and the species cannot be separated. Neither can they be separated in the second column with the exception of the range (B) of U. striatus and U. pisi.

Because of these intergradations of the ranges given in the literature, and the difficulty of applying them to delimit the species of the U. pisi group, it was decided to measure under constant conditions, the spores of all the species of the group from as many different localities as possible, and to apply statistical methods of analyses to these measurements.

(b) Morphological examination of specimens.

(i) Sources and preparation of material for spore measurements.

61 specimens of the U. pisi group were obtained from the herbarium of the Department of Plant Pathology at Cornell University, Ithaca, N.Y., U.S.A. Of these, 7 had very slight infections of only one or two seri, and preparations were not made of these. 24 further specimens were obtained from the herbarium of the Commonwealth Mycological Institute and preparations were made of all these. Most of the specimens examined had either uredo- or teleutospore stages, or both. A few had both pycnidia and aecidia and others only the latter. The specimens were collected in Europe, Asia and America, and determinations were in most cases by well known authorities such as Mayor, E, Fischer, E., Bisby, G. R., Wiltshire, S. P., Jensen, C., etc. Specimens of U. laburni were not examined.

Pieces of host material bearing seri were softened in 20% alcohol to render the host tissue less brittle and also to serve as a wetting agent for the mounting fluid. After 30 minutes to 1 hour, the tissue was removed and excess alcohol was absorbed by small pieces of filter paper. Fresh filter paper was used after each slide had been prepared. The spores were then transferred by needles from the sorus

to a drop of the following mounting medium:

Phenol	....	20 gms.
Lactic acid	....	20 c.c.
Glycerol	....	40 c.c.
Distilled water	...	20 c.c.

When fresh spores were used, they were mounted directly in this fluid. After each slide was prepared, the needles were carefully washed, then held in an alcohol flame until red hot. This ensured that the spores of different specimens would not be mixed. The slides were heated gently over an alcohol flame to reveal the germ pores and the sculpturing of the wall and also to induce turgidity. None of the spores appeared to be over turgid. In all, 94 slides were prepared.

(ii) Methods of measuring and of statistical analysis.

Measurements:

The measurements were made using a filar ocular micrometer with the scale arranged on a rotating drum. The microscope, with a tube length of 160 m.m. and a 2 m.m. oil immersion objective of 100 x magnification, was calibrated with a micrometer slide, and calibration charts were constructed. 10 divisions on the micrometer drum equalled 0.36 μ of the field viewed.

Table 16.

Source	Host	Length			Breadth				
		Range	M	Sd.	Range	M	Sd.	W.	P.C.
C.U.	Lathyrus pratensis	21-25	23.5	0.9	14-.9	16.3	1.4	2.6	4.3
"	" "	22-25	23.9	0.7	15.5-18.5	16.8	0.8	2.5	4.1
Local	" "	21-25.5	24.5	0.8	15.5-19	17.1	0.9	2.6	4.2
C.U.	" sylvestris	23-27	24.9	0.8	15.5-19	16.7	0.7	2.5	3.6
"	" "	24-28.5	25.9	1.0	16-19	17.5	0.6	2.5	3.5
I.M.I.	" "	25-28.5	25.3	1.08	17.5-20	19	0.65	2.7	3.8
"	" "	24.5-27	26	0.6	17.5-19.5	18.7	0.5	2.4	4.2
"	" "	25-27.5	26	0.7	17.5-20	18.3	0.7	2.8	4.4
C.U.	Pisum sativum	24-26.5	25.3	0.6	16.5-20	18.	0.9	2.4	4.6
"	" "	24-25.5	24.8	0.5	16.5-20.5	17.9	0.8	2.6	4.4
"	" "	25-28.5	26.7	0.95	17-19.5	18.3	0.6	2.7	4.7
I.M.I.	" "	24-28	26.2	0.83	17-20	19	0.8	2.8	4.4
"	" arvense	24-27	25.2	0.8	17.5-19.5	18.7	0.5	2.4	4.2

Table 16. U pisi. Statistical data on teleutospore measurements. Figures in microns.

C.U. = Cornell University herbarium; I.M.I. = Imperial Mycological Institute herbarium;

M. = Mean; Sd. = Standard deviation; W. = Wall thickness;

P.C. = Pore cap thickness; L - Length; B = Breadth.

Table 17.

Source	Host	Length			Breadth				
		Range	M	Sd.	Range	M	Sd.	W.	P.C.
C.U.	Oxytropis pilosa	20-23.5	21.8	1.1	15.5-19	17.4	0.8	1.8	2
"	" campestris	21.5-25.5	23.9	0.95	16-19.5	17.5	0.7	1.9	3.25
"	" montana	20-25	22	1.15	16-18.5	17.5	0.55	2.1	3.5
"	Astragalus alpinus	16.5-20.5	18.5	0.9	16.5-18.8	17.4	0.65	2.1	3.6
"	" glycyphyllos	21.5-26.5	24	1.15	15-18.5	16.9	0.9	2.2	2.95
I.M.I.	" "	21.5-25.5	23.6	0.8	16-18.5	17.6	0.55	2.5	3.5
"	" "	22.5-25	24	0.6	16-18.5	17.5	0.5	2.5	3.95
C.U.	" leontinus	20-24.5	21.8	1.2	15.5-18.5	16.7	0.6	2.2	3.25
Local	" danicus	20-25.1	22.8	0.8	15-18.5	16.8	0.7	2.3	3.1

Table 17. U punctatus. Statistical data on teleutospore measurements. Figures in microns.

Abbreviations as in table 16.

Tables 18 and 19.

Source	Host	Length			Breadth				
		Range	M	Sd.	Range	M	Sd.	W.	P.C.
C.U.	Medicago sativa	22-26	23.4	0.9	14.5-18.5	16.7	0.9	1.9	3.8
"	" "	19-23	20.9	0.8	14.5-18	16.3	0.5	2.2	2.9
"	" Lupulina	20-23.5	21.7	0.7	14.5-19	16.9	1.1	2.2	3.5
"	" "	22-25.5	23.8	0.7	13.5-17	15.9	0.7	2.3	3.7
"	" "	18.5-22.5	20.6	0.75	15-17.5	16.2	0.6	2.1	4.3
Local	" "	19.24.1	21.9	0.7	14-18.3	16.3	0.7	2.2	3.4
C.U.	" falcata	20-25	22.7	1.2	14-17	16.2	0.75	2.3	3.5
"	" varia	21-24	22.6	0.7	14.5-17.4	16.1	0.7	2.2	3.4
"	Trifolium arvense	20-24.5	22.4	0.8	14.5-18.5	16.8	0.9	2.5	4.2
"	" campestre	21-24	22.5	0.7	14-17.5	16.1	0.9	2.1	3.7

Table 18. U.striatus: Statistical data on teleutospore measurements. Figures in microns.

Abbreviations as in Table 16.

Source	Host	Length			Breadth				
		Range	M.	Sd.	Range	M.	Sd.	W.	P.C.
C.U.	Lotus corniculatus	22.5-25	24.1	0.5	15-18	17.5	0.75	2.1	3.1
"	" "	21.5-25.5	23.5	0.8	14.5-18	16.7	0.7	2.2	2.9
I.M.I.	" "	23.5-27	25	0.7	16.5-19.5	18.5	0.7	2.1	3.4
Local	" "	21.5-26	24.3	0.7	15-19.1	17.2	0.8	2.2	3.1
I.M.I.	" edulis	24-27.5	26.3	0.82	16-20	18.2	0.84	2.6	3.9

Table 19. U. loti. Statistical data on teleutospore measurements. Figures in microns.

Abbreviations as in Table 16.

Tables 20 and 21.

Source	Host	Length			Breadth				
		Range	M	Sd.	Range	M.	Sd.	W.	P.C.
C.U.	Vicia cracca	26.5-32	28.9	1.2	22-27.5	26.4	1.2	2.8	4.7
"	" "	27.5-32	29.6	1.15	21.5-24.5	22.9	0.7	2.8	4.5

Table 20. U. fischeri eduardii: Statistical data on teleutospore measurements. Figures in microns. Abbreviations as in Table 16.

Source	Host	Length			Breadth				
		Range	M	Sd.	Range	M.	Sd.	W.	P.C.
I.M.I.	Astragalus exscapus	22-25	23	0.72	16.5-19	18	0.7	2.3	3.4
C.U.	" "	20.5-24	22.6	0.8	16-20	17.9	0.9	2.3	3.9

Table 21. U. iordianus: Statistical data on teleutospore measurements. Figures in microns. Abbreviations as in Table 16.

Tables 22 and 23.

Source	Host	Length			Breadth				
		Range	M.	Sd.	Range	M.	Sd.	W.	G.P.
C.U.	Lathyrus pratensis	21.5-24.5	23.5	0.7	16.5-21	19	1.0	2.5	3-6(4)
"	" "	21-25	23.2	0.75	17-20.5	18.9	0.9	2.6	4-5(4)
"	" "	21.5-25.5	23.9	1.0	17-22	21.7	1.2	2.6	3-5(4)
Local	" "	21-24.5	23.4	0.7	17-21.5	19.1	0.9	2.7	3-5(4)
C.U.	Pisum sativum	22.5-26.5	24.3	0.95	17.5-22	20.3	1.0	2.5	4-6(4)
"	" "	21-25	23.4	0.9	17.5-21.5	19.3	0.95	2.3	3-5(3)
"	" "	20.5-26.5	23.9	1.7	18.5-23.5	22	1.24	2.4	3-5(3)

Table 22. U pisi: Statistical data on uredospore measurements. Figures in microns except G.P. = No. of germ pores. Other abbreviations as in Table 16. (in G.P. column.

Source	Host	Length			Breadth				
		Range	M.	Sd.	Range	M.	Sd.	W.	G.P.
C.U.	Oxytropis pilosa	20-25.5	23.3	1.4	17.5-22	20.5	0.8	2	3-4(3)
"	" montana	21.5-29.5	25.4	1.8	18.5-22	20	0.9	2.4	3-5(3)
"	" halleri	22.5-27	25.1	1.0	20-23.5	21.5	0.8	2.3	3-4(3)
"	Astragalus alpinus	20.5-24.5	22.1	0.95	17.5-21	19.3	0.85	2.1	3-4(3)
I.M.I.	" lamperti	25.5-28.5	27.2	0.61	20-22.5	21.6	0.55	2.8	4-6(4)
"	" pentaglottis	24.5-27.5	26.3	0.7	17.5-21.5	19.6	0.8	2.3	3-4(4)
Local	" danicus	21-26.5	24.9	0.8	18-22.5	20.5	0.8	2.2	3-5(4)

Table 23. U. punctatus: Statistical data on uredospore measurements. Figures in microns except in G.P. column. Other abbreviations as in Tables 16 and 22.

Tables 24 and 25.

Source	Host	Length			Breadth				
		Range	M.	Sd.	Range	M.	Sd.	W.	Q.P.
C.U.	Medicago sativa	22-25	23.4	0.6	16.5-19	17.8	0.6	2.2	3-4(3)
"	" "	21-24.5	22.5	0.8	16-19	17.3	0.6	2.2	3-4(3)
"	" "	20-24.5	22	0.95	16.5-21.5	18.3	1.15	2.2	3-4(3)
I.M.I.	" "	21.5-25.5	23.8	0.85	18.5-22.5	19.4	0.9	2.7	4-6(4)
"	" "	21-24.5	22.8	0.8	17-20	18.8	0.7	2.2	3-5(4)
C.U.	" lupulina	20-23.5	21.4	0.7	16.5-19.5	17.5	0.5	2.4	3-4(3)
"	" "	19.5-22.5	21.5	0.7	16-20	18	0.7	2.5	3-4(4)
I.M.I.	" "	21-24.5	22.9	0.8	17-19	18.1	0.7	2.6	3-5(4)
Local	" "	20-24.5	21.9	0.7	16.5-20	18.2	0.6	2.2	3-5(4)
C.U.	" falcata	22-25.5	23.9	0.6	14.5-20	17.5	1.0	2.2	3-4(4)
"	Trifolium campestre	20.5-24.5	22.5	0.9	18-21.5	20	0.8	2.2	3-4(4)

Table 24. U. striatus: Statistical data on uredospore measurements. Figures in microns except in Q.P.Column. Other abbreviations as in Tables 16 and 22

Source	Host	Length			Breadth				
		Range	M.	Sd.	Range	M.	Sd.	W.	Q.P.
C.U.	Lotus corniculatus	23.5-27.5	25	0.95	17.5-21.5	19.8	1.0	2.3	3-5(4)
I.M.I.	" "	24-27.5	25.6	0.8	19.5-22.5	21.1	0.7	2.6	4-5(4)
"	" "	23.5-27.5	26.5	0.7	20-24	22.1	0.2	2.8	3-5(4)
Local	" "	24-27.5	25.3	0.7	19-23	21	0.6	2.5	3-5(4)
I.M.I.	" uliginosus	24-27.5	26	1.0	20-23	21.9	0.7	2.6	4-5(4)

Table 25. U. loti: Statistical date on uredospore measurements. Figures in mocrons except in Q.P. column. Other abbreviations as in Tables 16 and 22.

Statistical methods.

From all specimens, random samples of 60 spores were selected for measuring. In some cases where the infection was slight and very few seri were present, samples of less than 60 but greater than 30 spores were used. All calculations were carried out in micrometer units and finally converted to microns.

The measurements of each character, e.g. length, breadth, etc., were assembled in groups of increasing magnitude with class intervals of 10 micrometer units (= 0.36 μ). From this data, the mean and the standard deviation were calculated by standard methods. The lowest and the highest measurement for each character was taken as the range of that character. It was then possible using these statistics to compare the different specimens within each species. The data for each species presented in tables 16 to 25, were based on an examination of 1,560 uredospores and 2,160 teleutospores involving some 14,880 individual measurements. An examination of the statistics presented in these tables reveals that (1) the ranges given for the various specimens within a species, have a large measure of agreement, and (2) the means of the measurements of the characters $\pm$ 2 standard deviations stand fairly near each other. The ranges presented in these tables were determined from

Tables 26 and 27.

Species	Length				Breadth						
	Range	M	S.E.M.	Sd	Range	M	S.E.M.	Sd.	W	P.C.	$\frac{L}{B}$(M)
U. pisi	21-28.5	25.5	0.05	1.3	14-20.5	18	0.05	1.2	2.6	4.2	1.42
U. punctatus	20-26.5	23	0.06	1.35	15-19.5	17	0.03	0.7	2.2	3.2	1.35
U. striatus	18.5-25.5	22.3	0.05	1.3	13.5-19	16.4	0.04	0.85	2.2	3.7	1.36
U. loti	21.5-27.5	24.9	0.08	1.3	14.5-20	17.5	0.07	1.1	2.3	3.3	1.42
U. fischeri eduardii	26.5-32	29.3	0.1	1.2	21.5-27.5	23.7	0.1	1.2	2.8	4.6	1.24
U. iordianus	20.5-25	22.8	0.08	0.9	16-20	18	0.07	0.8	2.3	3.7	1.26

Table 26. Statistical data for the teleutospores of each species. All specimens of each species examined; treated as one large sample. Figures in microns.
S.E.M. = Standard error of mean; Abbreviations as in Table 16.

Species	Length				Breadth						
	Range	M	S.E.M.	Sd.	Range	M	S.E.M.	Sd.	W.	G.P.	$\frac{L}{B}$(M)
U.pisi	20.5-26.5	24	0.07	1.15	16.5-23.5	19.5	0.07	1.4	2.5	3-6(4)	1.23
U.punctatus	20-29.5	25	0.1	2.1	17.5-23.5	203	0.07	1.2	2.3	3-6(3)	1.24
U. striatus	19.5-25.5	22.2	0.05	1.15	14.5-22.5	18.6	0.06	1.4	2.3	3-6(4)	1.19
U. loti	23.5-27.5	25.7	0.08	0.85	17.5-24	21.2	0.07	1.2	2.6	3-5(4)	1.21

Table 27. Statistical data for the uredospores of each species. All specimens of each species examined treated as one large sample. Figures in microns except in G.P. colomn. Abbreviations as in Tables 16 and 22.
S.E.M. = Standard error of mean.

specimens collected in similar localities as those described in the literature (tables 1 to 15), and a comparison of the data in the two sets of tables, illustrate the advantage of having a standard method of measuring the spore characters. The variation shown in the ranges given in tables 1-15 is no doubt partly due to the small sizes of the samples measured, e.g. Arthur (1929, p. 369) states that "The range of measurements given for spore sizes is customarily based on the average of some 10 or 12 individual spore measurements."

However, this part of the study is concerned more with the differences and similarities between species rather than with the variation within each species. Obviously to compare statistically one specimen with all other specimens examined in the group, would involve a great deal of calculation and it would be questionable if much value could be attached to the results. It was thus necessary to calculate statistics to represent each species in order to facilitate comparison. This was done by using the original measurements on which tables 16-25 are based. In this method all the measurements of the specimens of one species were treated collectively as one random sample of that species, and the necessary statistics were calculated for each character. The results of these calculations are presented in tables 26 and 27. A further column has been added where the mean (L)

is divided by the mean (B). This gives an indication of the shape of the spores, e.g. as the ratio mean (L)/mean (B). approaches unity, the shape of the spore tends to be spherical.

(iii) Lengths and breadths of spores.

In table 26 with the exception of U. fischeri-eduardii, the teleutospore ranges (L and B) of all species are almost the same. The means (L and B), however, show some differences except the mean (B) of U. pisi and U. jordianus which are identical, and the mean (L) of U. punctatus and U. jordianus have only a slight difference.

The ranges (L) and (B) for the uredospores are all of the same order of magnitude, though the range (L) of U. loti is somewhat higher. No uredospores were available for U. fischeri-eduardii and U. jordianus. The means (L and B) are all different.

To test whether these means were significantly different, the 'student t test' was applied in the following manner. The test was applied between the mean of one character, say mean (L), of one species e.g. U. pisi, and the mean of the corresponding character of all the other species. Then the mean (L) of U. striatus was tested with the mean (L) of the other species except U. pisi, since this

Table 28.

Species c.f. Column 2.	Species c.f. Column 1.	Teleutospores L	Teleutospores B	Uredospores L	Uredospores B
U. pisi	U.punctatus	30.75	16.39	8.3	7.98
" "	U.striatus	42.6	26.3	24.0	10.0
" "	U.loti	6.19	5.75	20.0	16.0
" "	U.fischeri eduardii	29.9	48.3	-	-
" "	U.iordianus	21.9	0.0	-	-
U.punctatus	U.pisi	30.75	16.39	8.3	7.98
" "	U.striatus	8.1	12.3	23.6	18.1
" "	U.loti	17.6	7.6	5.0	9.4
" "	U.fischeri eduardii	46.0	80.0	-	-
" "	U.iordianus	1.5	13.6	-	-
U.striatus	U.pisi	42.6	26.3	24.0	10.0
" "	U.punctatus	8.1	12.3	23.6	18.1
" "	U.loti	26.0	15.5	39.0	25.0
" "	U.fischeri eduardii	53.8	78.5	-	-
" "	U.iordianus	4.5	18.8	-	-
U.loti	U.pisi	6.19	5.75	20.0	16.0
" "	U.punctatus	17.6	7.6	5.0	9.4
" "	U.striatus	26.0	15.5	39.0	25.0
" "	U.fischeri eduardii	31.0	50.0	-	-
" "	U.iordianus	15.8	4.6	-	-
U.fischeri eduardii	U.pisi	29.9	48.3	-	-
" " "	U.punctatus	46.0	80.0	-	-
" " "	U.striatus	53.8	78.5	-	-
" " "	U.loti	31.0	50.0	-	-
" " "	U.iordianus	47.7	35.7	-	-

Table 28 (Contd.)

Species c.f. Column 2.	Species c.f. Column 1.	Teleutospores		Uredospores	
		L	B	L	B
U. jordianus	U.pisi	21.9	0.0	-	-
" "	U.punctatus	1.5	13.6	-	-
" "	U.striatus	4.5	18.8	-	-
" "	U.loti	15.8	4.6	-	-
" "	U.fischeri eduardii	147.7	135.7	-	-

Table 28. Results of the calculation of 'significant difference' between the characters L and B of the different species. Values above 1.96 indicate a significant difference.

was already calculated. Then the mean (L) of U. punctatus was tested with the means (L) of the remaining three species and so on. The significant difference was thus calculated for all characters of both spore types. The results, calculated at a 5% level of significance, are presented in table 28. In this table any value above 1.96 indicates a significant difference.

From this table it will be seen that there is a significant difference in all cases with two exceptions. The exceptions are the breadths of the teleutospores of U. pisi and U. jordianus, and the lengths of the teleutospores of U. punctatus and U. jordianus. Although there is no significant difference between these characters mentioned, the other characters of the spore i.e. the length in the case of U. pisi c.f. U. jordianus and the breadth in U. punctatus c.f. U. jordianus, differ significantly. Since both the lengths and the breadths of the spore types must be considered together, it can be concluded on the evidence presented that all of the species are significantly different from one another and that the samples measured came from different populations.

(iv) Wall thickness of spores.

From the few measurements of wall thickness of the

teleutospores given in the literature, there appears to be little variation. The measurements when given vary from 1 to 2 μ. In the case of the uredospores, however, there appears to be distinct differences in wall thickness between certain species. For example, 1.5-2 μ is given for U. striatus and U. fischeri-eduardii; 1.5-2.5 μ for U. pisi, U. punctatus, U. jordianus and U. laburni; and 2.5-3.5μ is given for U. loti.

The measurements of the wall thickness of both the uredospores and teleutosperes made in this investigation, do not show the differences indicated in the literature. The data are summarised in table 27. In this table the wall thicknesses are in all cases between 2.2 and 2.8 μ. These measurements, as already pointed out, were determined using an oil immersion objective of 100 x magnification. At such high magnifications the accuracy of the measurements is greatly increased, and it is suggested that the difference in the measurements between those recorded in the literature and those presented in table 27, may be due to this factor.

(v) Pore cap thickness of teleutospores.

Apart from general descriptions of the pore cap given in the literature such as 'not pointed' (Schroeter 1871), there is only one measurement given and that is 3 μ for

U. pisi by Sydow (1904-12).

In tables 16-21, the thicknesses of the pore cap are given for all the specimens examined by the writer. These data are summarised in table 26.

These figures show that there are three groups of thicknesses:- (1) U. pisi and U. fischeri-eduardii, (2) U. striatus and U. jordianus and (3) U. punctatus and U. loti. However it is doubtful whether this character can be used to any great extent for separating the species of the group, although there is considerable difference between the thickness of the pore cap of U. punctatus (3.2 μ) and U. fischeri-eduardii (4.6 μ).

(vi) Germ pore number and distribution.

From the literature (tables 8-14) it appears that only U. loti can have as few as 2, although it may have as many as 5 germ pores. The other members of the group with one exception, have numbers between 3 and 6, U. punctatus for example having constantly 3-4. U. jordianus has 6-8 germ pores, and this character has been used for keying out this species. The germ pores are randomly distributed in all species.

The data obtained by the writer are presented in tables 22-25 and a summary is given in table 27. No uredospores of U. f. eduardii and U. jordianus were available.

It will be noted that U. loti has 3-5 germ pores in all the specimens examined by the writer. Otherwise the reported results agree well with those obtained from the literature.

(vii) Sculpturing of the teleutospore wall.

Reference has already been made to the difficulty of describing this character and the confusion which has resulted from this in the literature. It is not proposed to discuss this point further.

The uredospores are not dealt with in the following, as observation showed that all the species had spores which were echinulate to the same degree - or nearly so - and in fact the only difference which could be noted between the species was the sizes of the spores.

The observations made on the teleutospores were from the slides prepared for the spore measurements. A critical examination of the spores on these slides showed that even within a species there was considerable variation of the markings on the walls. In order to illustrate this feature, photographs were taken with the phase-contrast microscope. By this means any thick part of the wall, e.g. punctations, would show up darker in a print (Plates I-VI, figs. 1-31). It will be noted that in some of these photographs, only part of the wall is shown, and this is due to the natural curvature of the spores. All

photographs were taken at the same magnification and were enlarged by the same factor, so that the sizes of the punctations etc. can be compared. All exposures were approximately the same. A green Wratten filter was used in taking these photographs as the spores were orange coloured.

U. punctatus. Plate I, figs. 1-6.

A comparison of figures 1-6, all of which are U. punctatus, shows that there is a variation in the type of punctations or warts on the walls. The most common type is illustrated in figs. 1 and 2. In fig. 3 the punctations are not so marked as in figs. 1 and 2, and they appear to be more randomly scattered. The warts in fig. 4 show a tendency to anastomose and become lengthened, and this is further illustrated in figs. 5 and 6. The last figure (6), bears a resemblance to some of the U. striatus figures.

U. striatus. Plate II, figs. 7-12.

It can be seen from the photographs that here as in U. punctatus (Plate I), there is a gradation from a definite punctate sculpturing, to one where the punctations become extended for a considerable length of the spore. In fig. 7, the punctations are quite distinct. The tendency

to anastomose is shown in fig. 8. Figs. 9 and 10, show much larger punctations or warts and these are quite separate. It was only possible to photograph two of the long striate punctations shown in fig. 11 - the spore on the right. Fig. 12 shows one long striation running at least half the length of the spore.

U. loti. Plate III, figs. 13-17.

Fig. 13 shows two spores with contrasting wall sculpturing. The one on the left has long striate punctations, whereas the punctations of the other spore are small and rounded. The warts shown in fig. 14 are quite distinct and large. Fig. 15 shows an intermediate stage between distinct punctations and definite striations, and this latter is further illustrated in figs. 16 and 17. In this species, as in the first two described, there are a great number of intermediate types between a distinctly punctate and a striate wall. This latter character is similar to that existing in U. striatus (Plate II).

U. laburni. Plate III, fig. 18.

Only one photograph was taken of this species as there appeared to be little variation in the wall markings. The punctations are quite distinct and resemble those of U. loti, fig. 14.

U. pisi. Plate IV, figs. 19-24. Plate V, figs. 25-26.

In this species there is no tendency for the punctations to anastomose or become striate. However, there is a definite gradation from an almost smooth epispore, to one where the punctations are quite distinct. Some of the latter resemble U. punctatus, U. fischeri-eduardii and U. jordianus. There are all types of intermediate sculpturing from almost smooth to definitely punctate.

In fig. 19, the wall is almost smooth, though there is a trace of punctations on the left hand side of the upper spore in the photograph. The punctations are very faint in fig. 20 but become more definite in figs. 21 and 22, and certainly more pronounced in figs. 23-26.

U. jordianus. Plate V, figs. 27-29.

In fig. 27, the punctations are small and distinct, and they resemble some cases of U. pisi. The warts in fig. 28 are much larger and tend to be arranged in linear order. Some anastomosing of the punctations is shown in fig. 29 c.f. U. striatus, fig. 7, (Plate II).

U. fischeri-eduardii Plate V, fig. 30. Plate VI, fig. 31.

There are only two photographs of this species. In fig. 30, the punctations are much smaller and more

Text fig. 1.

U. pisi

U. fischeri-eduardii

U. jordianus

U. punctatus

U. laburni

U. loti

U. striatus

Text fig. 1. Diagrammatic representation of the sculpturing of teleutospore walls.

numerous than in fig. 31. The latter resembles some of the types found in U. punctatus, (Plate I) and the former is not very different from U. pisi (Plates IV and V).

It is obviously unnecessary to produce photographs of all the types of spore wall markings found in the examination of the specimens already mentioned, to show that variation exists. Sufficient evidence has been presented, which, in the mind of the writer, indicates clearly that there are variations in the sculpturing of the wall within any one species. As has been pointed out, there are also numerous similarities between the species. The conclusion is, that there is no constant wall configuration for any one species of this group. This agrees well with Kobel's (1921) findings. He had examined a large number of specimens of U. punctatus in the Mayor herbarium, and found that the sculpturing varied within this species. The markings of the wall could be classified into two or more groups, but he was reluctant to form new species or varieties on this basis because of the existence of numerous intermediate types.

This tendency of the group to show a gradation from a smooth to a striate epispore is illustrated diagramatically in text fig. 1.

Thus it can be seen, that the sculpturing of the wall alone provides insufficient evidence to delimit the species.

IV. PHYSIOLOGICAL DATA.

Introduction.

One of the most interesting characters of the rust fungi is their ability to select certain host plants on which to carry out their life cycles. This aspect of the rusts has been given a great deal of study in recent years, especially those rusts parasitic on economic crop plants.

The early mycologists recognised that species of rust differed from each other in the hosts which they parasitised. They also noted that rust species which were similar morphologically, were found to parasitise closely related host plants. The fact that these fungi were obligate parasites no doubt influenced their concept of the species.

Further modifications of the species concept was brought about by the discovery that even within morphologically similar groups i.e. species, there existed smaller specialised groups which differed in the hosts they selected. The result has been that some investigators have based their concept of the species mainly on morphological characters, while others have inclined to place greater emphasis on the host specialisation. As

Arthur (1929), points out, "The subdivisions, varieties, races, forms etc., of some taxonomists often became species in the treatment by others." These differences in concept should be borne in mind when making comparisons of the species.

Rusts which are grouped together on a morphological basis, generally have a wide host range. The hosts for the two phases of the life cycle of heteroecious rusts are usually members of widely separated families e.g. species of the genus Gymnosporangium usually have their aecidial stage on the Rosaceae, while the other stage of development is mostly restricted to members of the Cupressineae. Apart from the widely separated host relationship of the two phases, there is often considerable difference in the range of each phase. Some species for instance have a wide range for the uredo phase and a restricted range for the aecidial phase, while the converse is true of other species. The former case is illustrated by Puccinia graminis Pers. where the aecidia are developed on a few species of Berberis and Mahonia while the uredo stage occurs upon 98 species of 35 genera of the Poaceae (Arthur & Frome, 1920). P. aristidae Tracy, on the other hand, exhibits the reverse situation. Its uredo stage occurs on one species of grass, Distichlis spicata while the aecidia

occur on hosts belonging to 90 species of 64 genera in 24 families (Arthur & Frome, 1920). Other cases exist where both phases have wide or narrow ranges.

Rusts may not only be distinguished by the species of host on which they develop, but can be further separated by their reaction to certain varieties of such host species. For example, some of the races of P. graminis can be further segregated by their differences in behaviour to certain cultivated varieties of cereals (Stakman & Levine, 1922). These more specialised types are usually referred to as physiological forms. Such forms have been found in other cereal rusts, but little or no work has been done on other species. The narrow specialisation of these physiological forms can be developed further, for certain of them have been found to be selective even within a variety of agronomic wheat (Mains & Jackson, 1926). All these physiological forms however, can only be distinguished when suitable differentiating hosts are employed.

From the foregoing, the dynamic nature of the relationship existing between host and parasite, is apparent. Because of this, it is obvious that great care must be exercised to ensure that both the host and the parasite are pure lines before any progress can be made in studying this aspect of the rusts. This is perhaps of

more importance in the case of the heteroecious rusts, for here the possibility of obtaining hybrids of the rust is greater, due to their heterothallic nature.

In such studies, other factors must be taken into consideration. Such factors as temperature and humidity of the environment, the vigour of the host and its nourishment, etc., all play a part in the host-parasite relationship. Generally speaking, conditions which modify the metabolism of the host have a more or less corresponding effect upon the development of the rust. Consequently when inoculation experiments were carried out in the present study these physical factors were controlled as far as possible.

To attempt the separation of a wild species of rust into its races and physiological forms, if these were present, would involve many years' work. For example, in certain heteroecious rusts, a year must elapse between the time of inoculating the aecidial host and the production of aecidia. In some cases a period of two years is necessary for the development of the aecidia. This is the general situation obtaining in the Uromyces pisi group, and because of this and the non-germinability of the teleutospores, the determination of the aecidial hosts and the specialisation on these hosts, was not studied.

Concerning the uredo hosts of the U. pisi group, it was originally intended that all the host plants recorded in the literature for the individual species of the group would be tested, (1) as hosts for, and (2) to determine if biological races existed in, the local specimens of the rusts. A summary of the literature, however, revealed that the number of hosts recorded was considerable, e.g. Oudemans (1921) alone, gives 29 host species in 3 genera for U. striatus and 21 host species in 2 genera for U. punctatus. A series of inoculation experiments carried out with such large groups of hosts and with closely related rusts, would have involved a serious risk of contamination, and would have required more greenhouse space than was available. It was decided, therefore, that since U. punctatus was a new record for Britain and had only been found locally on Astragalus danicus, inoculation experiments would be carried out with this rust with a view to determining the uredo host range, and its degree of specialisation, if any. Consequently, in the following pages, U. punctatus is discussed in greater detail than the other members of the group. Some inoculation experiments were also carried out with U. pisi and U. loti.

A critical examination of the literature was conducted to discover the host range and extent of specialisation of each species of the group. An attempt has been made to check all the references occurring in the literature and most of these have been read. Unfortunately, some of the original works, especially those of German origin, were unobtainable due to the war, and where these are quoted in the present study, abstracts have been consulted, or the reference has been cross-checked with other independent authors. A list of synonyms of each species has also been compiled. Following Wilson & Bisby (1954), the names of the British hosts occurring in the literature have been altered in accordance with, 'The Flora of the British Isles' (Clapham, Tutin & Warburg, 1952), without comment. For non-British host species, Index Kewensis has been followed.

(a) Uromyces punctatus Schroet.

(i) Literature review.

The rust was first recorded under the name Uredo astragali Opiz; α astragali glycyphylli by Opiz in 1852. Saccardo, (1873), transferred it to the genus Uromyces and gave it the name Uromyces astragali (Opiz) Sacc. Meanwhile, Schroeter (1869) had described a new species of

rust which he named U. punctatus Schroet. n.s.

In his original published record (1852), Opiz gives the host as Astragalus glycyphyllos. Schroeter (1869), added A. nigrescens and A. ponticus to the host list and placed it in his group of Hemi-uromyces as aecidia had not yet been found. Some years later (1885), he suggested that it may be an imperfect form of a Hetero-uromyces species. Passerini (1877), followed Schroeter's nomenclature and gave A. glycyphyllos and Onobrychis sativa as hosts. In 1880, Winter reported that he had found young pycnidia and aecidia along with uredospores and teleutospores of U. punctatus Shroet on Oxytropis campestris and Phaca alpinum. Although the pycnidia and aecidia were too undeveloped to give a description, he suggested that this species of rust might be synonymous with the autoecious Aecidium carneus Nees. Saccardo in his Sylloge Fungorum (1888) retained the name he gave this rust (U. astragali (Opiz) Sacc), and gives the following host list: A. glycyphyllos, A. danicus, A. hypoglottis, A. testiculatus, A. melilotus, A. arenarius, A. ponticus, A. apaca, A. nigrescens, A. alopecurieides, A. leucophaea and Onobrychidis viciifolia. He includes under U. astragali a variety lupini (B. et C.) occurring on Lupinus spp. but Hariot (1892) points out that this variety does not belong here.

Although Schroeter (1869) had named the rust U. punctatus, in 1889 he adopts the name given by Saccardo (1873) and quotes U. punctatus as a synonym. No new hosts were reported. According to Hariot (1892) the rust may be found on Colutea species, though he gives no host species or reasons for including this genus in the host list. A. austriacus was reported as a host by Vestergren (1902).

The heteroecious nature of the rust was demonstrated by Jordi (1903) after a series of culture experiments. Using aecidia occurring on Euphorbia cyparissias he inoculated species of Oxytropis, Astragalus and Lotus, and obtained uredo-infections on O. montanus, O. campestris, O. glabra; A. cyclyphyllos and L. corniculatus. Although he was aware of Winter's (1880) record of young aecidia and pycnidia occurring with uredospores and teleutospores on Oxytropis species, he concluded that U. astragali (Opiz) Sacc. was heteroecious and the aecidial stage was found on Euphorbia cyparissias. It should perhaps be mentioned here that in the spring (1953) the writer has seen what looked like young aecidia developing on A. danicus, and which agreed with Winter's (1880) limited description. On further culturing, the orange spots on the leaves eventually gave rise to fruiting structures of Pseudopeziza species.

Jordi continued his work on the leguminous rusts and published his results in 1904. In this paper he described two new species and emended a third. These were Uromyces euphorbiae-astragali, U. euphorbiae-corniculati and U. astragali (Opiz) Jordi emend. The first mentioned species is the one under discussion in this section and the other two are dealt with in the sections on U. loti (Kirch.) Blytt and U. jordianus Bubak, respectively. Jordi added A. lapponica to the host list.

Fischer (1904) gives the same host list and used the new name given by Jordi, but suggests that O. halleri and Phaca alpinum are possible hosts. The heteroecious nature of the rust was confirmed by Bubak in 1904. He inoculated Euphorbia cyparissias with over-wintered teleuto material and obtained aecidial infections. The following year he pointed out that the name U. euphorbiae-astragali Jordi n.s. was not in accordance with the rules of priority and consequently renamed it U. astragali (Opiz) Sacc. For U. astragali Jordi emend. he proposed the name U. jordianus Bubák, (1905).

A doubtful additional aecidial host, E. gerardiana, was given by Mueller in 1907. The following year both Bubak and Trotter each record only E. cyparissias for the aecidial host. Bubak added O. pilosa and Trotter,

A. sicalis to the uredo host list. It is interesting to note that Trotter (1908) uses the full specific epithet of Opiz (1852) i.e. U. astragali-glycyphylli. Sydow (1904-12), apart from P. alpinum does not give the specific names of the hosts. His list is:- Astragalus spp; Onobrychidis spp; Oxytropis spp; and Halleris spp. and adds that E. cyparissias is the aecidial host.

After a series of experiments, Treboux (1912) established that E. virgata could act as an aecidial host. He inoculated A. hypoglottis, A. cicer, A. ponticus, A. cruceatus, A. hamosus, A. falcatus, A. thianschanicus A. viciaefolius, and A. virgatum with aecidiospores from E. virgata and obtained positive infections. There was no biological specialisation among this group of hosts. Species of Trifolium, Medicago, Lotus, Caragana and Melilotus were not infected.

Constantineanu (1920), apart from other Astragalus species already mentioned above, included A. onobrychidis var. microphylli and A. galegiformis in his host list. He claimed A. falcatus as a new host though it had already been reported by Treboux (1912). A new host, A. vesicarius was added by Savulescu (1941-2). Guyot (1951) reports that the rust is common on A. glycyphyllos and other Astragalus species but rare on A. leontinus in France.

New hosts given by Guyot are A. aristatus, A. callichrous, A. deinacanthus and A. sanctus.

The majority of references to this rust in the literature are under the name U. astragali (Opiz) Sacc. and this is based on the International Rules of Nomenclature prior to 1950. An interpretation of the rules since 1950, would allow the rejection of this name (Saccardo 1873), and the acceptance of the name given by Schroeter (1869). Thus U. punctatus Schroet. is considered to be the valid name since the specific epithet is not based on the genus Uredo.

U. punctatus is very rare in Britain and was first recorded on A. danicus by Macdonald (1949) for the St. Andrews (Fife) district. Then, only uredospores were found, but subsequent investigation has revealed both uredo- and teleutospores. Wilson & Bisby (1954) give Angus as an additional locality for the rust.

(ii) Host list of U. punctatus.

The following list of host plants for U. punctatus has been compiled from the literature. British species, according to Clapham, Tutin & Warburg (1952) are marked with a !. No distinction has been made between hosts determined by culture experiments and the naturally

occurring hosts. The host species are given in the left hand column and the reference to each host in the right hand column.

Species of Host.	Cited by:-
Oxytropis montana D.C.	Jordi (1903, 1904), Kobel (1921), Fischer (1904), Oudemans (1921).
" campestris (L) D.C.	Jordi (1903, 1904), Kobel (1921), Fischer (1904), Winter (1880), Trotter (1908), Oudemans (1921).
" glabra D.C.	Jordi (1903, 1904), Fischer (1904).
" halleri Bunge	Kobel (1921), Fischer (1904), Oudemans (1921).
" pilosa D.C.	Kobel (1921), Bubàk (1908), Trotter (1908), Constantineanu (1920), Oudemans (1921).
" lapponica Gray	Fischer (1904), Jordi (1904), Oudemans (1921).
Astragalus glycyphyllos L.	Jordi (1903, 1904), Saccardo (1873, 1888), Treboux (1912), Kobel (1921), Passerini (1877), Schroeter (1869, 1875, 1889), Bubàk (1904, 1908), Fischer (1904), Guyot (1951), Savulescu (1941-2), Trotter (1908), Constantineanu (1920), Oudemans (1921).
" hypoglottis L.	Treboux (1912), Saccardo (1888), Schroeter (1875).
" cicer L.	Treboux (1912), Bubàk (1908), Oudemans (1921).
" ponticus Pall.	Treboux (1912), Schroeter (1869, 1889), Saccardo (1888), Savulescu (1941-2), Constantineanu (1920), Oudemans (1921).

Astragalus cruciatus	Lk.	Treboux (1912).
" hamosus	L.	Treboux (1912), Rayss (1951), Oudemans (1921).
" falcatus	Lam.	Treboux (1912), Constantineanu (1920).
" thianschanicus	Bunge	Treboux (1912).
" viciaefolius	D.C.	Treboux (1912).
" virgatus	Pall.	Treboux (1912), Constantineanu (1920).
" utahensis	Torr. & Gray	Kobel (1921).
" purshii	Dougl.	Kobel (1921).
" danicus	Retz. !	Kobel (1921), Saccardo (1888), Oudemans (1921), Macdonald (1949), Wilson & Bisby (1954).
" austriacus	Jacq.	Kobel (1921), Vestergren (1902), Bubàk (1908), Oudemans (1921).
" multiflorus	Gray	Schroeter (1869, 1889), Saccardo (1888).
" onobrychis	L.	Kobel (1921), Bubak (1908), Trotter (1908), Constantineanu (1920), Oudemans (1921).
" var. microphylli	Bess.	Constantineanu (1920).
" testiculatus	Pall.	Saccardo (1888), Oudemans (1921).
" melilotoides	Pall.	Saccardo (1888).
" arenarius	L.	Saccardo (1888), Schroeter (1889), Oudemans (1921).
" opacus	?	Saccardo (1888), Schroeter (1889).
" alopercurioides	L.	Saccardo (1888), Schroeter (1889), Trotter (1908), Oudemans (1921).

Astragalus leucophaeus Sm.		Saccardo (1888), Schroeter (1889), Oudemans (1921).
"	leontinus Wulf.	Guyot (1951).
"	depressus L.	Oudemans (1921).
"	vesicarius L.	Savulescu (1941-2).
"	siculis Riv.	Trotter (1908), Oudemans (1921).
"	aristatis L'Herit	Guyot (1951).
"	callichrous Bois	Rayss (1951).
"	deinacanthus Bois	Rayss (1951).
"	monspessulanus L.	Oudemans (1921).
"	sanctus Bois	Rayss (1951).
"	galegiformis L.	Constantineanu (1920).
"	fruticosus Willd.	Constantineanu (1920).
"	frigidis Gray	Schroeter (1875).
"	alpinus L. !	Winter (1880a, b), Kobel (1921), Fischer (1904), Trotter (1908), Oudemans (1921).
Onobrychis viciifolia Scop. !		Passerini (1877), Saccardo (1888).
Euphorbia cyparissias L. !		Kobel (1921), Bubàk (1904, 1908), Trotter (1908), Fischer (1904), Sydow (1904-12), Migula (1910, 1917) Oudemans (1921).
"	virgata W. & K. !	Treboux (1912).
"	gerardiana Jacq.	Mueller (1907).

From the above list, it will be seen that the rust has been recorded for 6 species of Oxytropis, 35 species and one

variety of Astragalus, 1 species of Onobrychis, and 3 species of Euphorbia. Of the Oxytropis species recorded, 2 are British, and of the 35 species of Astragalus only 3 are British. Onobrychis viciifolia is the only British species of this genus and E. cyparissias and E. virgata are not common in Britain.

Most of the hosts mentioned above have a wide distribution in Europe. A few species are found in S.W. Asia extending as far east as Palestine and some have been recorded for Morocco in N. Africa. U. punctatus follows this distribution generally, and though most of the records are for Central Europe, this is no doubt due to the greater number of mycologists in that region.

(iii) Synonyms of U. punctatus.

In compiling the list of synonyms of U. punctatus, it has been impossible to read every reference in the literature for reasons already given. However, the list has been so constructed that reference can be made to the actual citations of the names given. These citations have been verified.

The rust has been referred to as U. punctatus only by Schroeter (1869, 1870, 1871, 1875), Passerini (1877), Winter (1880), Arthur (1934) and Wilson & Bisby (1954).

In the list given below, some of the names in the left hand column have been cited as synonyms by the authors in the right hand column, while in other cases, the names in the left hand column have actually been used by these authors, but have since become synonyms, e.g. Saccardo (1888) described the rust under the name U. astragali (Opiz) Sacc., but gives Uredo astragali Opiz as a synonym and these are both listed below as synonyms without any differentiation. All other authors who have quoted these names either as a valid name or as a synonym are consequently cited opposite the names, and so on.

Name of fungus.	Cited by.
Uredo astragali Opiz	Bubàk (1908), Sydow (1904-12), Saccardo (1888), Oudemans (1921).
U. astragali glycyphylli Opiz	Sydow (1904-12), Trotter (1908).
U. astragali ciceris Opiz	Sydow (1904-12).
U. oxytropidis Opiz	Sydow (1904-12).
U. oxytropidis Peck.	Hariot (1892).
U. acuminata Kirch.	Bubàk (1908), Sydow (1904-12), Oudemans (1921).
U. astragali Lasch.	Oudemans (1921).
U. apiculata Strauss	Oudemans (1921).
U. apiculatus Lev. f. astragali Pass.	Passerini (1877).
Caeoma apiculatus Schlect.	Oudemans (1921).

C. uredo-leguminosarum Mart.	Oudemans (1921).
Uromyces oxytropidis Knze.	Hariot (1892), Sydow (1904-12), Oudemans (1921).
U. cytisi Schroet.	Hariot (1892).
U. astragali (Opiz) Sacc.	Saccardo (1873, 1883), Jordi (1903), Kobel (1921), Vestergren (1902) Guyot (1951), Bubak (1904), Sydow (1904-12), Savulescu (1941-2), Constantineanu (1920), Migula (1910, 1917), Oudemans (1921), Treboux (1912), Schroeter (1889).
Uromyces euphorbiae-astragali Jordi	Jordi (1904), Bubak (1908), Fischer (1904), Trotter (1908), Migula (1910, 1917).
U. punctatus Schroet.	Oudemans (1921), Trotter (1908), Sydow (1904-12), Saccardo (1888), Migula (1910, 1917).
U. phacae Thum.	Oudemans (1921).
U. genistae-tinctoriae Wint.	Winter (1880), Oudemans (1921).
U. astragali-glycyphylli (Opiz) Sacc.	Trotter (1908), Oudemans (1921).
U. appendiculatus Lev.	Oudemans (1921).
U. genistae Schroet.	Oudemans (1921).
U. laburni Fuck.	Oudemans (1921).
Nigredo punctata Arth.	Arthur (1912, 1934).

(iv) Determination of uredo host range of U. punctatus at St. Andrews.

The following experiments were conducted in the greenhouse to determine the range of uredo hosts of U. punctatus

growing locally, and also to investigate if biological specialisation existed.

(v) Source of host plants.

Seeds of 27 species of Astragalus, 9 species of Lathyrus, 6 species of Oxytropis, 5 species of Cytisus (Sarothamnus), 4 species of Genista, 4 species of Vicia, 2 species of Pisum, 5 species of Medicago, 2 species of Lotus, 8 species of Euphorbia, and one species each of Anthyllis, Trigonella, Onobrychis and Caragana, were obtained from the following Botanic Gardens:- Lyons (France), Dublin (Ireland), Chelsea (England), Ottawa (Canada), Montpellier (S. France), Liege (Belgium), Leyden (Holland) and Coimbra (Portugal) and also from the Bailley Hortorium (Ithaca, New York, U.S.A.). Plants of Astragalus danicus and Lotus corniculatus were obtained from seed from the Botanic Garden (St. Andrews), or seeds collected from wild plants, or in some cases the naturally growing plants were dug up and potted. These latter were not used for inoculations until after 3 weeks.

Seeds from all the species were sown at regular intervals to ensure that the plants would be of the same approximate age. 3½" pots were used and the number of plants per pot varied from 1 to 5. The plants were kept in a cold frame until at least two leaves were showing when

they were brought into the greenhouse and left for 4 days. Pots were labelled as to species, date of sowing, date of inoculation, and source.

(vi) Method of inoculations and results.

Plants to be inoculated were examined carefully with a hand lens to ensure that they were free from rust. Only those which appeared in a vigorous condition and were well established, were selected for experiment. Usually plants in 2 pots were inoculated and one other pot was used as a control. Where germination of the seed was poor and fewer pots available only one pot was inoculated. But in all cases at least one pot was kept as a control.

The experimental plants were placed in an inoculation chamber and atomised with sterile distilled water. The chamber consisted of a wooden frame covered with two layers of fine cheese cloth. Prior to inoculation the cheese cloth was soaked with water, and all windows and doors of the greenhouse were closed to prevent draughts. The plants were then inoculated by atomising with a concentrated suspension of uredospores in sterile distilled water and placed in the incubation chamber. (fig. 34). The bottom of the chamber was covered with a sheet of corrugated iron on top of which was a 4" layer of Sphagnum moss and a 4"

layer of peat soil. This was thoroughly soaked with water prior to incubation, and ensured a continual saturated atmosphere within the chamber.

The temperature of the greenhouse was thermostatically controlled at 22°C, and the temperature within the chamber was recorded with a maximum and minimum thermometer. The minimum temperature recorded was 18°C and the maximum 24°C. The incubation period lasted two days and the temperature within the chamber was read hourly between 9 a.m. and 10 p.m. each day. The mean temperature over the two days was 21.5°C. To test the germinability of the uredospores, hanging drops of the spore suspension were made in Van Tieghem cells and placed in the incubator along with the inoculated plants.

After incubation the plants were removed from the chamber and arranged in the greenhouse.

Experiment 1.

The inoculum was obtained from naturally infected A. danicus plants on the golf course. It was considered that this was a reasonably pure culture since the rust had been growing on A. danicus in this locality for a number of years and the aecidial stage was absent. Inoculation was carried out on 29/6/53 and the plants were examined on 12/7/53.

The results are given in table 29.

Table 29.

Species	Source	Pot No.	Inoculated (I) or Control (O)	Result
Astragalus danicus Retz.	J	1	I	+ve
"	J	2	I	+
"	J	3	I	+
"	J	4	O	-ve
"	J	5	O	-
scorpioides Pourr.	A	1	I	-
"	A	2	I	-
"	A	3	O	-
hamosus L.	A	4	I	+
"	A	5	I	-
"	A	6	O	-
suleatus L.	A	7	I	-
"	A	8	I	-
"	A	9	O	-
onobrychis L.	A	10	I	-
"	A	11	I	-
"	A	12	O	-
chlorostachys Lindl.	B	1	I	-
"	B	2	I	-
"	B	3	O	-
"	B	4	O	-
pentaglottis L.	B	5	I	-
"	B	6	I	-
"	B	7	O	-
vicioides Grah.	B	8	I	-
"	B	9	I	-
"	B	10	O	-
cicer L.	C	1	I	-
"	C	2	I	-
"	C	3	O	-
laeteus H. & S.	D	1	I	+
"	D	2	I	-
"	D	3	O	-
granatensis ?	D	4	I	+
"	D	5	I	+
"	D	6	O	-
lusitanicus Lam.	D	7	I	+
"	D	8	I	-
"	D	9	O	-
alpinus L.	E	1	I	+
"	E	2	I	-
"	E	3	O	-
caryocarpus Ker-Gawl.	G	1	I	+
"	G	2	I	-
"	G	3	O	-

Table 29 (Contd.)

Species	Source	Pot No.	Inoculated (I) or Control (O)	Result
Astragalus falcatus Lam.	D	10	I	-
"	D	11	I	-
"	D	12	O	-
alopecurioides L.	E	4	I	+
"	E	5	I	-
"	E	6	O	-
monspessulanus L.	A	13	I	-
"	A	14	I	-
"	A	15	O	-
"	A	16	O	-
robbinsii Gray	B	11	I	-
"	B	12	I	-
"	B	13	O	-
glycyphyllos L.	H	1	I	+
"	H	2	I	+
"	H	3	O	-
"	H	4	O	-
adsurgens Pall.	E	7	I	-
"	E	8	I	-
"	E	9	O	-
sesameus L.	F	1	I	-
"	F	2	O	-
purshii Dougl.	G	4	I	-
"	G	5	I	-
"	G	6	O	-
iridactylis ?	G	7	I	-
"	G	8	I	-
"	G	9	O	-
caespitosus Gray	G	10	I	-
"	G	11	I	-
"	G	12	O	-
lecontianus Wulf.	C	4	I	-
"	C	5	O	-
Oxytropis halleri Bunge	C	6	I	-
"	C	7	I	-
"	C	8	O	-
erebroleuca Bunge	C	9	I	-
"	C	10	I	-
"	C	11	O	-
campestris (L). D.C.	C	12	I	-
"	C	13	I	-
"	C	14	I	-
"	C	15	O	-

Table 29 (Contd.)

Species	Source	Pot No.	Inoculated (I) or Control (O)	Result
Oxytropis deflexa D.C.	E	7	I	-
"	E	8	I	-
"	E	9	O	-
splendens Dougl.	E	10	I	-
"	E	11	I	-
"	E	12	O	-
Lathyrus pratensis L.	K	1	I	-
"	K	2	I	-
"	K	3	O	-
sylvestris L.	C	16	I	-
"	C	17	I	-
"	C	18	O	-
latifolius L.	H	5	I	-
"	H	6	I	-
"	H	7	O	-
aphaca L.	K	4	I	-
"	K	5	I	-
"	K	6	O	-
sativus L.	F	3	I	-
"	F	4	I	-
"	F	5	O	-
odoratus L.	H	8	I	-
"	H	9	I	-
"	H	10	O	-
hirsutus L.	C	19	I	-
"	C	20	I	-
"	C	21	O	-
heterophyllus L.	A	17	I	-
"	A	18	I	-
"	A	19	O	-
maritimus Bigel	H	11	I	-
"	H	12	I	-
"	H	13	O	-
Sarothamnus scoparius (L) Wimm	C	22	I	-
"	C	23	I	-
"	C	24	O	-
Cytisus nigrescens L.	C	25	I	-
"	C	26	I	-
"	C	27	O	-
hirsutus L.	C	28	I	-
"	C	29	I	-
"	C	30	O	-

Table 29 (Contd.)

Species	Source	Pot No.	Inoculated (I) or Control (O)	Result
Cytisus purpureus Scop.	H	14	I	-
"	H	15	I	-
"	H	16	O	-
albus Link.	H	17	I	-
"	H	18	I	-
"	H	19	O	-
Genista tinctoria L.	H	20	I	-
"	H	21	I	-
"	H	22	O	-
pilosa L.	C	31	I	-
"	C	32	O	-
sagitallis L.	C	33	I	-
"	C	34	O	-
anglica L.	H	23	I	-
"	H	24	I	-
"	H	25	O	-
Vicia cracca L.	B	14	I	-
"	B	15	I	-
"	B	16	O	-
sativa L.	B	17	I	-
"	B	18	I	-
"	B	19	O	-
sepium L.	B	20	I	-
"	B	21	I	-
"	B	22	O	-
orobeides Wulf.	C	35	I	-
"	C	36	O	-
Pisum sativum L.	C	37	I	-
"	C	38	O	-
arvense L.	C	39	I	-
"	C	40	I	-
"	C	41	O	-
Medicago sativa L.	D	13	I	-
"	D	14	I	-
"	D	15	O	-
lupulina L.	H	26	I	+
"	H	27	I	+
"	H	28	O	-
hispida Gaert.	A	20	I	-
"	A	21	I	-
"	A	22	O	-
falcata L.	C	42	I	-
"	C	43	I	-
"	C	44	O	-

Table 29 (Contd.)

Species	Source	Pot No.	Inoculated (I) or Control (O)	Result
Medicago arabica (L). All.	K	7	I	-
"	K	8	I	-
"	K	9	O	-
Trigonella foenum-graecum L.	M	29	I	-
"	M	30	I	-
"	M	31	O	-
Anthyllis vulneraria L.	B	23	I	-
"	B	24	I	-
"	B	25	O	-
Lotus uliginosus Schk.	C	45	I	-
"	C	46	O	-
corniculatus L.	J	6	I	-
"	J	7	I	-
"	J	8	O	-
Caragana arborescens Lam.	K	10	I	-
"	K	11	I	-
"	K	12	O	-
Onobrychis viciifolia Scop.	M	32	I	-
"	M	33	I	-
"	M	34	O	-

Table 29. Results of inoculation experiment using uredospores of U. punctatus on A. danicus. References: A = Lyons, B = Dublin, C = Chelsea, D = Coimbra, E = Ottawa, F = Montpellier, G = Bailley Hort. M = Liege, K = Leyden, J = St. Andrews, Botanic Garden. J = seeds collected from local wild plants, I = Inoculated, O = Control.

From this experiment, 9 species of Astragalus and one species of Medicago were infected. Regarding the latter infection, the sori were very small and only a few uredospores developed. There were only 2 sori in all of the 10 plants. All the control plants remained healthy.

Experiment 2.

Healthy A. danicus plants were inoculated with the spores of each of the positively infected plants of Experiment 1 by transferring the spores on the point of a scalpel and then covering the inoculated plant with a bell jar lined with wet filter paper. Positive infections were obtained in all cases except the Medicago rust which was not in sufficient quantity.

Experiment 3.

Experiment 1 was repeated using new plants and also some which had been controls in Experiment 1. The plants were inoculated in the same manner as Experiment 1 on 15/7/53 and were examined on 30/7/53. The following species were infected: A. danicus, A. scorpioides, A. hamosus, A. alopecuroides, A. pentaglottis, A. lacteus, A. graustonis, A. lusitanicus, A. alpinus, M. lupulina and Caragana arborescens.

Again the infection on Medicago was very weak. Only one sorus developed on Caragana and the leaf bearing it

eventually dropped off and was lost. Of the 9 Astragalus species infected in this experiment, A. scorpioides and A. pentaglottis were not infected in Experiment 1, whereas A. caryocarpus and A. glycyphyllos were not infected in this experiment but were infected in Experiment 1. All the control plants remained healthy.

Experiment 4.

Uredospores, from the plants successfully infected in Experiment 3 were inoculated on to healthy A. danicus as in Experiment 2. All inoculations gave positive results. The inoculations using the Medicago and the Caragana rust were not attempted.

Experiment 5.

Experiment 1 was repeated again except that species of Lathyrus, Lotus, Pisum, Vicia, Sarothamnus, Cytisus, Genista and Onobrychis were not included. The plants were inoculated on 4/8/53 and examined on 20/8/53.

The following species became infected: A. danicus, A. hamosus, A. lacteus, A. granstonis, A. lusitanicus, A. alpinus, A. caryocarpus, A. alopecurioides, A. glycyphyllos, A. pentaglottis and M. lupulina. Caragana was not infected.

Experiment 6.

The rusts on the above plants were inoculated on to A. danicus and all, including M. lupulina, gave positive

results after 12-16 days.

It thus appears that the plants successfully infected in Experiment 5, can act as hosts for U. punctatus found at St. Andrews. This number of species is considerably less than the total species recorded in the literature as hosts, and would suggest some sort of specialisation. It is possible that the rust at St. Andrews is one race of U. punctatus, but this could not be tested since viable material of the rust from another locality was not available.

Experiment 7.

A series of smaller experiments was carried out to determine if the local U. punctatus was composed of races. The experiments were conducted as in Experiment 2 since the amount of inoculum was small. This factor also limited the number of inoculations done.

The rust on	A. hamosus	was inoculated on to		A. alpinus
"	"	"	A. alpinus	"	"	A. hamosus
"	"	"	A. lacteus	"	"	A. caryocarpus
"	"	"	A. caryocarpus	"	"	A. lacteus
"	"	"	A. granstonis	"	"	A. alopecurioides
"	"	"	A. alopecurioides	"	"	A. granstonis
"	"	"	A. lusitanicus	"	"	A. glycyphyllos

The rust on A. glycyphyllos was inoculated on to A. lusitanicus

" " A. pentaglottis " " A. lusitanicus

All 9 plants became infected. Since the pairings of these plants for cross inoculations were selected at random, and since all gave positive results it is concluded that it is highly improbable that the rust occurring at St. Andrews is composed of races.

The plants from various sources (A-K, table 29) appeared to show no difference in susceptibility to infection by U. punctatus. For example, A. hamosus in Experiment 2 was from Coimbra and in Experiments 1 and 3 from Lyons. In all three experiments it was successfully infected.

(vii) Life cycle of U. punctatus at St. Andrews.

General description of the rust.

The summer crop of uredosori usually makes its appearance in June. The sori occur on the leaves, stems, and petioles of the infected plants as reddish-brown pustules. On the leaves, they are usually found on the upper surface, though they are not uncommon on the lower surface. There is no regular distribution of sori on the leaves. On a heavily infected leaf there may be as many as ten sori. Two pustules which are close together may coalesce, so forming one large

sorus. The average diameter of a sorus is 800 μ and the range is 400-1,200 μ. On the stem and petioles the sori are elongated and often attain a length of 4 m.m. When very young the sorus is covered with epidermis which is ruptured at an early stage. Towards the end of the summer (Oct.-Nov.) the teleutospores are produced. These spores are not found in such great abundance as the uredospores, and generally, an examination of a sorus in December reveals a number of uredospores mixed in with the teleutospores. The Deuteromycete, Darluca filum has been found in increasing abundance during the past four years both in the sori and around them.

Mode of perpetuation.

As already noted from the literature, the aecidial stage of U. punctatus occurs on Euphorbia cyparissias. This plant was not found in the immediate neighbourhood, although it is in cultivation in some gardens at least ½ mile away. The only record in Fife is for the Dunfermline district some 30 miles away (Young 1935). It was thus necessary to determine by what means the rust was able to perpetuate itself.

The naturally infected plants were visited almost fortnightly from September till June in 1950-51 and 1952-53, and from July till March 1953-54. Young plants of A. danicus appeared in May of each year and were always healthy. After

January most of the plants died off, but some which were protected by grass, etc., remained green and bore a few uredosori. It was thus established that the rust was present all the year round. Some of the old withered leaves also bore uredosori though often the spores were empty.

It was decided to test the viability of these spores.

Experiment 8.

In March, 1951, healthy A. danicus plants were inoculated, and became infected after 14 days. In every month of the year 1952-53, infected A. danicus plants were collected and spores from this material were used to inoculate A. danicus plants in the greenhouse. A positive result was obtained for each experiment. Between January and March 1954, four successful inoculations were made on healthy A. danicus using uredo material collected during that period. In all these experiments, the inoculations were made by transferring the spores on the point of a scalpel and then incubating the plant under a bell jar lined with wet filter paper for two days.

From the above experiments there can be no doubt that the rust can perpetuate itself by uredo infections alone. However, the possibility that the fungus is systemic on A. danicus and thus overwinters in the stock, cannot be ruled out.

Experiment 9.

To investigate this point, leaves and stems bearing sori were collected during November-April 1950-51 and 1952-53, fixed in Formal-Acetic-Alcohol and embedded in paraffin. These were sectioned and stained with (1) Cotton blue in Lactophenol and (2) Congo red. On examination under the microscope no trace of mycelium was found beyond the hyphal mass below each sorus. The first uredosori occurring on young A. danicus plants in June 1951 and 1953 were similarly treated. The only hyphae seen were those below the sori.

It is thus reasonable to conclude that the rust is not systemic and that it can perpetuate itself from year to year by means of uredospores.

(viii) Germination of teleutospores.

During the year 1949-50, numerous attempts were made to germinate both fresh and overwintered teleutospores.

Experiment 10.

Hanging drop cultures of the spores were made in tap, sterile distilled water, 10%, 20% and 30% cane sugar solutions and incubated at 22°C. The cultures were examined regularly, but after 3 days there was no evidence of teleutospore germination. Similar results were obtained with whole leaves of A. danicus bearing teleutosori which were incubated at

Table 30.

Slide No.	Date of collection.	Storage.	Germination	
			Tel.	Ured.
1 - 3	18/11/52	Tin	-ve	+ve
4 - 6	18/11/52	Refrig.	-	+
7 - 9	12/12/52	Tin	-	+
10 - 12	12/12/52	Refrig.	-	+
13 - 15	7/1/53	Tin	-	+
16 - 18	7/1/53	Refrig.	-	+
19 - 21	10/2/53	Tin	-	+
22 - 24	10/2/53	Refrig.	-	+
25 - 27	5/3/53	Tin	-	+
28 - 30	5/3/53	Refrig.	-	+
31 - 33	3/4/53	Tin	-	+
34 - 36	3/4/53	Refrig.	-	+

Table 30. Germination of teleutospores in hanging drop cultures of sterile distilled water. Tin - storage in tin outside. Refrig. = refrigerator.

22°C in a petri dish lined with moist filter paper. On one occasion, overwintered teleutospores were transferred to a piece of moist filter paper on a slide, placed in a petri dish moist chamber, and incubated at 22°C. After 24 hours the slide was examined but only one teleutospore had germinated

A further attempt was made to germinate the teleutospore in April 1953.

Experiment 11.

Collections of rusted A. danicus were made between November 1952 and April 1953, and stored either in a tin kept outside or in the refrigerator at 0-1°C. The material was brought into the lab and first soaked in sterile distilled water in solid watch glasses for 1½ hours, and then allowed to dry. After drying in the lab for 3-4 hours the material was again soaked and hanging drop cultures were made. Each collection of rust was kept separate. 3 cultures were made from each collection, 2 of which were incubated at 22°C and the other at room temperature (13-17°C). The spores were examined after 6, 12, 24, 36, and 48 hours but none of the teleutospores had germinated although uredospores present showed normal germination after 12 hours. (table 30).

Numerous cases are reported in the literature where germination of fungus spores has been stimulated by some chemical agent (Noble 1923, 1924, Brown 1922, Duggar 1901,

Maneval 1924, Raeder & Bever 1931, Thiel & Weiss 1920, Ling 1940, Cotter 1940, Emerson 1948 and others). These agents were sometimes distillates of the host plant (Brown 1922), extracts of germinating seeds of the hosts or tissue extracts (Noble 1924, Whitehead 1920-1, and others), some of the essential oils (Noble 1923, 1924), or other chemicals such as Benzaldehyde, Citric acid and Butyric acids (Noble 1924, Theil & Weiss 1920, Raeder & Bever 1931, Emerson 1948 and others). From this literature it appears that Benzaldehyde, iso- and n- Butyric acids and Citric acid were the most successful stimulating agents. Most of these authors favour a presoaking period in water varying from 1 hour to several days prior to the actual test.

It was consequently decided to test the effect of these substances on the teleutospores of U. punctatus in an endeavour to induce germination.

In March, 1954, the following experiments were carried out using teleuto infected A. danicus collected in November and December 1953 and in January, February and March 1954. The collections were stored in tins outside.

Experiment 12.

Benzaldehyde: The teleuto material was soaked overnight in sterile distilled water (st. dist. water). The next morning it was blotted dry and spores were transferred to hanging drops

of the following Benzaldehyde dilutions in st. dist. water: $1:10^2$; $1:10^3$; $1:10^4$; $1:10^5$; $1:10^6$ and st. dist. water alone. The cultures were then sealed and incubated at 22°C. For each collection of rust, 18 cultures were made i.e. 3 for each of the 5 dilutions and 3 for the st. dist. water. In all 90 cultures were prepared.

Observations were made after 6, 15, 22, 36 and 48 hours but none of the teleutospores had germinated. Between the 6 and 15 hour observations it was noted that germination of the uredospores present had taken place except in the $1:10^2$ and $1:10^3$ dilutions. The cultures were examined after 4 days with the same results.

Experiment 13.

The above experiment was repeated 3 days later but using only 2 cultures for dilution i.e. 60 cultures in all. On examination none of the teleutospores had germinated.

Experiment 14.

Citric acid. 3 experiments were carried out with this substance. The teleuto material was soaked for 15 minutes in a 1% solution of Citric acid in st. dist. water (Thiel & Weiss 1920). 3 hanging drop cultures in st. dist. water were then made from each collection of rust and incubated at 22°C. The cultures were examined regularly up to 3 days

after the commencement of the experiment, but no teleutospores had germinated.

Experiment 15.

Dilutions of Citric acid were made as for the Benzaldehyde experiment (12). 2 cultures were prepared for each dilution. The material was treated in the same manner as in Experiment 12 above. During the following 4 days the cultures were examined at intervals. No teleutospores had germinated.

Experiment 16.

Experiment 16 was repeated with the same results.

Experiments 17 & 18.

The benzaldehyde experiment (12) was repeated exactly but using n-Butyric acid, (Experiment 17) and iso-Butyric acid, (Experiment 18) for the dilutions. In both cases no teleutospores had germinated up to 3 days.

It will be noted that in the above series of experiments, both fresh and overwintered teleutospores were used. In view of this and the consistent negative results, it would appear that these spores have lost their ability to germinate.

(ix) The aecidial stage.

Despite the fact that the teleutospores could not be induced to germinate, a series of inoculation experiments were carried out on Euphorbia cyparissias.

Experiment 19.

On 6/4/50, overwintered teleuto-infected material of A. danicus was soaked in water and then spores were transferred on the point of a scalpel to the young shoots of the developing E. cyparissias. Other Euphorbias were inoculated by placing pieces of teleuto-infected leaves of A. danicus between the leaves on the buds of Euphorbia. The potted Euphorbias were then placed in a dish of water and covered with a bell jar lined with moist filter paper which dipped into the water. The incubation period lasted 48 hours. After this period the plants were kept in a cool greenhouse.

The inoculated plants were examined in May 1950 and in the spring of the following years 1951, 1953 and 1954. At no time was there any trace of developing pynidia or aecidia.

Experiment 20.

In April 1950, healthy Euphorbia plants were obtained from the writer's garden, some 40 miles away. Some of these

were planted amongst the naturally infected A. danicus in the two areas where the rust had been found (the New Golf course and the Sand Dunes). The other Euphorbias were inoculated as in Experiment 20 above.

All Euphorbias were examined as in Experiment 20 above with the same result. Those planted on the golf course had been destroyed presumably by weed killer in the spring of 1952.

Experiment 21.

Healthy Euphorbias were again inoculated in March and April 1951 as in Experiment 20 above. Up to March 1954 there was no sign of infection.

Experiment 22.

Inoculations were again carried out on healthy Euphorbias in March, April and May 1953 by the same method. There was no trace of infection on 12/4/54.

From the above experiments it appears that U. punctatus has lost its ability to infect the aecidial host.

(x) Discussion and conclusions.

In the results and observations which have just been presented, the following facts concerning the life cycle of U. punctatus at St. Andrews have been established.

(1). The uredospores are present all the year round and are capable of infecting A. danicus regardless of the time of year they were collected. The teleutospores are not produced in great abundance.

(2). The rust is not systemic on A. danicus.

(3). The teleutospores could not be induced to germinate under a variety of conditions and stimulants, with the exception of a single spore.

(4). The aecidial host, E. cyparissias, is absent from the immediate vicinity of the naturally infected A. danicus.

(5). The aecidial host planted amongst the infected A. danicus on the golf course and sand dunes in 1950, showed no signs of aecidial infection up to 1954.

(6). Inoculations of E. cyparissias consistently failed to produce infection.

(7). The local U. punctatus is homogeneous.

Such deviations of the life cycle of U. punctatus at St. Andrews, from the life cycle described in the literature, are not unknown. Guyot (1937) for example records that in N. France the heteroecious leguminous rusts are able to overwinter by means of the uredospores. He came to this conclusion because of the absence of the aecidial hosts in the same regions. It is not clear from this paper, however, whether an attempt was made to determine if the rust

was systemic on the uredo hosts.

Concerning the difficulties in germinating the teleutospores, Lamb (1943) reports that Puccinia prostii which had been imported into the Botanic Garden (Edinburgh) on Tulipa spp. produced an abundance of teleutospores, but constant efforts to induce germination of these spores met with failure. Wilson (1948), remarking on this case, suggests that when the teleutospores are produced in the climate of Edinburgh they are incapable of germinating.

In the case of U. punctatus at St. Andrews it appears that the absence of the aecidial host has led to an increased production of uredospores - and these throughout the year - with a corresponding reduction in the number of teleutospores produced. This is considered by the writer to be of greater consequence in accounting for the above deviations, than the climatic factors as suggested by the work of Johansen (1886), Magnus (1893), Fischer (1904), Arthur (1920) and Jørstad (1940). These authors suggest that a rust producing all its spore forms in southern latitudes tends to shorten its cycle in northern regions with increased production of teleutospores. Most of this work has been based on observations of autoecious species and few heteroecious species have been discussed.

At St. Andrews it seems that in the absence of the

aecidial host, the rust has gradually adapted itself to the uredo host and in consequence the teleutospores have become redundant. This suggestion is supported by the fact that although E. cyparissias was introduced amongst the infected A. danicus plants, no aecidial infection has taken place. It may well be, of course, that climatic factors are of greater consequence here. In connection with this it is interesting to note that Grove (1913) records only uredo and teleutospores for U. acetosae adding that the aecidial stage is unknown in Britain. This rust is widely distributed in this country. In 1934, however, Grove & Chesters report finding the aecidial stage in the south of England. This find, according to these authors, was after a particularly warm spring and they conclude that the production of the aecidia "must have some connection with the weather."

The major physical factors operating at St. Andrews which probably affect the rust are, the cool winds from the sea keeping the temperature low; the high moisture content of the atmosphere; the traces of salt in the atmosphere and in the soil, and probably the great numbers of hours of sunshine.

Thus it is concluded that with U. punctatus at St. Andrews the main causes of the non-germinability of the teleutospores and the failure to infect E. cyparissias are

due to (1) the absence of the aecidial host over a period of time leading to the adaptation of the rust to the uredo host with an increase in the numbers of uredospores, and a corresponding reduction in the number of teleutospores produced, and (2) various climatic factors.

(b) Uromyces pisi (Pers. ex D.C.) Wint.

(i) Literature review.

There is a large number of references to this species in the literature, and in view of this only papers which deal with experimental determinations of hosts or heteroecism, will be discussed. Other papers which give only a list of hosts are given in the host list and citations at the end of this section.

The rust was first recorded by Persoon (1801) as Uredo appendiculata β pisi sativa. De Candolle referred to it first (1810) as Puccinia pisi and later (1815) as Uredo pisi. In his Enumeratio Fungorum (1860), Fuckel gives a 'forma pisi' under Uromyces appendiculatus Tul. and a 'forma lathyri' under U. apiculatus Lev. There can be no doubt that these two forms belong here.

At that time the rust was only known in its uredo stage, and although the aecidial stage existed it was described as a separate species, usually in the genus

Aecidium. It was Schroeter (1875), who first demonstrated the connection between the two phases and suggested that there was at least two specialised forms in the uredo phase, one on Lathyrus pratensis and one on Pisum sativum. Cooke (1878-9) states that the aecidial stage was unknown in Britain, and Karsten (1879), reports that it had not been seen in Finland. In 1892, Klebahn inoculated various legumes with aecidiospores from Euphorbia esula, and obtained uredo infections on P. sativum while all the other legumes gave a negative result. He remarks that these results were not conclusive as certain precautions had not been taken.

Biologic forms were discovered by Jordi (1903, 1904) after much culture work. Aecidiospores of E. cyparissias produced uredo infections when inoculated on to Vicia cracca. Uredospores on L. pratensis when inoculated on to P. sativum and V. cracca, produced infection only in the case of P. sativum, but in another experiment uredospores on P. sativum would not infect L. pratensis. Teleutospores on L. pratensis were able to produce aecidia on E. cyparissias. Later (1904), he states that the aecidia of these two forms (on V. cracca and L. pratensis) deformed the leaves of E. cyparissias in a different manner. Although the form on V. cracca was eventually named as the species U. fischeri-eduardii by Magnus (Fischer 1904) it is considered that a

form of U. pisi still exists on this host.

Klebahn (1904), apart from E. esula, records E. dulcis, E. verucossa, E. gerardiana, E. virgata, and E. lucida, as aecidial hosts, but was uncertain if the aecidia on these host species belonged to any one form of the rust. He had not determined if the teleutospores could overwinter. In the same year, Fischer notes that a specimen of U. pisi on L. pratensis which he examined showed slight variations in sculpturing and size of the teleutospores from one on L. latifolius. In connection with this it should be mentioned that the writer has examined a species of rust on L. latifolius from the Cornell Herbarium. The specimen was labelled U. pisi (Pers) Wint. and was determined by E. Mayor, and from the spore measurements and sculpturing the writer has assigned it to the species U. lathyri-latifolii Guyot (1938). It is probable that Fischer's specimen also belongs here. Fischer lists a number of host plants some of which he considers doubtful and in need of confirmation. Among the latter are the aecidial hosts given by Klebahn (1904).

Buckheim (1922) carried out a series of experiments in which the form of U. pisi on L. pratensis would not infect several species of Vicia. The following year he confirmed E. esula as an aecidial host.

Moekrke (1927), gave a summary of the literature and concluded that although only two forms of the rust had been established, there was the possibility of a third, since in Jordi's (1903, 1904) experiments the form on P. sativum would not infect L. pratensis. He states that this would need confirmation by experiment. He separates the two established forms by the manner in which they deform the aecidial host E. cyparissias.

Guyot (1938-9) carried out a series of inoculation experiments with U. pisi and concluded that at least three forms exist:- one on P. sativum and P. arvense, one on L. pratensis, and one on P. arvense, P. sativum and L. aphaca. This was confirmed by further experiment (Guyot, 1939).

Thus from the literature it can be stated that on the Continent U. pisi is heteroecious; aecidial hosts are species of Euphorbia, mainly E. cyparissias; the uredo stage occurs on species of Lathyrus, Pisum and Vicia and at least three biologic forms have been described.

In Britain, the rust is comparatively rare though it has been recorded for numerous districts:- Grove (1913), Wilson (1934), Macdonald (1949), Mayfield (1935), Brambley et al. (1946), Moore (1933-42) and Wilson & Bisby (1954). In these reports the host is either L. pratensis or P. sativum. Wilson & Bisby (loc. cit.) state that the aecidial stage on

E. cyparissias has been recorded but that these reports are doubtful. However, both Coombe (1953), and Robertson (1953), state that aecidia of U. pisi have been found on E. cyparissias in W. Suffolk.

(ii) Host list of U. pisi.

The hosts recorded in the literature are listed below with their citations. British species of hosts are indicated thus !.

Euphorbia cyparissias L. !	Coombe (1953), Robertson (1953), Guyot (1938-9, 1939, 1951), Migula (1910, 1917), Persoon (1801), Constantineanu (1920), Schroeter (1875, 1889), Winter (1880), Savulescu (1941-2), Moehrke (1927), Fuckel (1860), Saccardo (1879), Fischer (1904), Sydow (1904-12), Tischler (1922), Beauverie (1923), Bubàk (1908).
esula L. !	Klebahn (1892, 1904), Migula (1910, 1917), Schroeter (1889), Fischer (1904), Sydow (1904-12), Buckheim (1923), Petrak (1920), Bubàk (1908).
lucida W. & K.	Klebahn (1904), Migula (1910, 1917), Schroeter (1889), Fischer (1904), Sydow (1904-12).
virgata W. & K.	Klebahn (1904), Migula (1910, 1917), Fischer (1904), Sydow (1904-12), Bubàk (1908).
dulcis L. !	Klebahn (1904), Saccardo (1879).
verucossa Lam.	Klebahn (1904), Beauverie (1923).

Euphorbia gerardiana	Jacq.	Klebahn (1904), Fuckel (1860).
Lathyrus pratensis	L. !	Petrescu (1926), Hariot (1908), Constantineanu (1920), Winter (1880, 1884), Guyot (1938-9, 1939), Jordi (1903, 1904), Moehrke (1927), Greville (1824), Schroeter (1889), Trotter (1908), Fischer (1904), Buckheim (1922, 1923), Persoon (1877), Bubák (1908), Karsten (1879), Brambley et al (1945), Wilson (1934), Macdonald (1949).
tuberosus	L. !	Petrescu (1926), Constantineanu (1920), Schroeter (1875, 1889), Winter (1880, 1884), Klebahn (1904) Fischer (1904).
nissolia	L. !	Constantineanu (1920), Klebahn (1904), Schroeter (1889), Fischer (1904), Buckheim (1922, 1923).
heterophyllus	L.	Sydow (1904-12), Buckheim (1923).
gergori	Parl.	Buckheim (1923).
articulatus	L.	Buckheim (1922, 1923).
latifolius	L.	Constantineanu (1920), Fischer (1904), Sydow (1904-12).
sylvestris	L. !	Schroeter (1875, 1889), Winter (1880, 1884), Klebahn (1904), Savulescu (1941-2), Trotter (1908), Fischer (1904), Buckheim (1923), Passerini (1877), Bubák (1908), Karsten (1879).
odoratus	L. !	Fischer (1904), Buckheim (1923).
cicera	L.	Trotter (1908), Sydow (1921, 1921a) Buckheim (1923), Passerini (1877).
montanus	Bernh. !	Buckheim (1923).
vernus	Bernh.	Buckheim (1923).

Lathyrus sativa L.	Guyot (1951), Schroeter (1875), Winter (1880, 1884), Trotter (1908) Fischer (1904), Passerini (1877), Bubàk (1908), Karsten (1879).
aphaca L. !	Passerini (1877), Guyot (1938-9, 1951), Schroeter (1875), Savulescu (1941-2), Trotter (1908), Buckheim (1923).
tingitanus L.	Guyot (1951).
Pisum sativum L. !	Guyot (1939, 1951), Rayss (1951), Petrescu (1926), Hooker (1836), Schroeter (1875, 1898), Winter (1880, 1884), Fuckel (1869-70), Moore (1934-42), Guyot (1938-9), Klebahn (1892, 1904), Savulescu (1941-2), Moehrke (1927), Trotter (1908), Fischer (1904), Sydow (1904-12), Buckheim (1922), Saccardo (1873), Passerini (1877), Bubàk (1908), Karsten (1879), Mayfield (1935-7), Wilson & Bisby (1954).
arvense L.	Winter (1880, 1884), Guyot (1938-9), Savulescu (1941-2), Fischer (1904), Sydow (1904-12), Buckheim (1922), Passerini (1877).
Vicia cracca L. !	Hariot (1908), Schroeter (1875, 1889), Winter (1880, 1884), Guyot (1938-9), Jordi (1904), Moehrke (1927), Trotter (1908), Fischer (1904), Karsten (1879).
cassubica L.	Winter (1880, 1884), Fischer (1904).
tenuifolia Roth.	Klebahn (1904), Schroeter (1889), Fischer (1904).
hirsuta (L) Gray !	Trotter (1908).
sativa L. !	Trotter (1908), Buckheim (1923).
patagonica Hook.	Fischer (1904).
Cicer arientinum L.	Passerini (1877).

(iii) Determination of uredo host range of U. pisi at St. Andrews.

As already mentioned, the St. Andrews records (Macdonald 1949) are on L. pratensis. The following experiment was conducted to determine which form of U. pisi was present.

Experiment 23.

Fresh uredo material of U. pisi on L. pratensis was collected at St. Andrews in mid July 1953, and inoculated on to the following plants by the method of Experiment 1. In all cases, control plants were used.

L. pratensis L.	L. hirsutus L.
L. maritimus Bigel.	L. heterophyllus L.
L. sylvestris L.	Vicia cracca L.
L. latifolius L.	V. sativa L.
L. aphaca L.	V. sepium L.
L. sativa L.	V. orobioides Wulf.
L. odoratus L.	Pisum sativum L.
	P. arvense L.

The plants were examined 15 days after inoculation but only L. pratensis was infected. All controls remained rust free.

Experiment 24.

Experiment 23 was repeated using some new plants and some of the controls of the last experiment.

Only L. pratensis became infected. All controls were healthy.

From these experiments it appears that U. pisi at St. Andrews is narrowly specialised on L. pratensis.

(iv) Synonyms of U. pisi.

The following list gives most of the synonyms for U. pisi. Some of the earliest ones have been omitted.

Aecidium cyparissiae D.C.	De Candolle (1815), Saccardo (1879) Plowright (1889), Oudemans (1921).
Ae. euphorbiae Pers.	Kickx (1867), Fuckel (1860), Persoon (1801), Hooker (1836), Schroeter (1875), Oudemans (1921).
Ae. euphorbiae Gmel.	Guyot (1951), Bubak (1908), Oudemans (1921), Fuckel (1860), Saccardo (1853, 1873), Winter (1884), Schroeter (1875), Passerini (1877).
Ae. euphorbiarum D.C.	De Candolle (1815), Oudemans (1921).
Caeoma aecidium euphorbiae Nees.	Oudemans (1921).
C. euphorbiae Nees.	Oudemans (1921).
C. euphorbiarum Lk.	Oudemans (1921).
C. apiculatum Schlecht.	Oudemans (1921).

C. appendiculatum Schlecht.	Oudemans (1921).
C. appendiculosum Lk.	Oudemans (1921).
Uredo pisi D.C.	De Candolle (1815), Oudemans (1921)
U. pisi Strauss	Strauss (1810), Oudemans (1921).
U. fabae Pers.	Greville (1824) p.p.
U. appendiculata pisi sativa Pers.	Persoon (1801), Bubàk (1908), Trotter (1908), Schroeter (1884), Strauss (1810), Oudemans (1921).
U. appendiculosa Berk.	Hooker (1836).
lathyri Belly.	Hariot (1892).
Puccinia pisi D.C.	De Candolle (1815), Schroeter (1875), Wilson & Bisby (1954).
Aecidiolum exanthematum Unger.	Saccardo (1879), Oudemans (1921), Passerini (1877).
Uromyces pisi (Strauss) de B.	Karsten (1879).
U. pisi (Pers) de B.	Migula (1910, 1917), Hariot (1892, 1908), Klebahn (1892, 1904), Bubàk (1908), Fischer (1904 a & b), Saccardo (1888), Schroeter (1889), Trotter (1908), Guyot (1938-9, 1951), Moehrke (1927), Rayss (1951).
U. pisi (D.C.) de B.	Saccardo (1873).
U. pisi de B.	Mayfield (1935-7), Cooke (1878-9), Passerini (1877), Fuckel (1869-70), Schroeter (1875).
U. lathyri Fuck.	Saccardo (1873), Trotter (1908), Schroeter (1875, 1884), Passerini (1877).
U. appendiculatus Tul. f. pisi Fuck.	Fuckel (1860).

Uromyces apiculatus Lèv. f. lathyri Fuck.		Fuckel (1860), Oudemans (1921).
"	appendiculatus Lèv.	Kickx (1867), Oudemans (1921).
"	apiculatus Lèv.	Kickx (1867), Oudemans (1921).
"	pisi (Strauss) Schroet.	Schroeter (1875).
"	pisi (Pers) Wint.	Petrak (1920), Sydow (1921, 1921a), Winter (1880, 1884), Buckheim (1922, 1923), Savulescu (1941-2), Constantineanu (1920), Petrescu (1926), Oudemans (1921), Brambley et al. (1945).
"	pisi Wint.	Moore (1933-42), Macdonald (1949), Wilson & Bisby (1954).

The name of this species is based on Uredo appendiculata β pisi sativa Persoon (1807), Puccinia pisi D.C. (1815) and Uromyces pisi (Pers) Winter (1880, 1884).

(c) Uromyces loti (Kirch.) Blytt.

(i) Literature review.

The rust was first described by Kirchner (1856) on Puccinia loti and is referred to as such by Saccardo (1888). In 1896, Blytt transferred it to the genus Uromyces and Saccardo (1912) cites this species as Uromyces loti Blytt. However, since the same specific epithet had been used by Kirchner (loc. cit.) the full citation should be U. loti (Kirch.) Blytt.

As mentioned in the section on U. punctatus, Jordi (1903, 1904) carried out a series of experiments with aecidia upon Euphorbia cyparissias. In these works he described three new species of rusts, one of which was U. euphorbiae-corniculati Jordi, which could infect Lotus corniculatus. He demonstrated that the rust was heteroecious and its aecidial host was E. cyparissias. Fischer (1904) cites the new name given by Jordi and suggests that apart from L. corniculatus the uredo stage may be found on other species of Lotus, though he does not list them. Under U. loti he refers to a rust on Vicia tenuifolia found by Magnus (1890) and states that it may belong here. (Magnus, in 1907 subsequently assigned it to the species U. viciae cracca Const.)

Bubak (1908) adds L. tenuifolius to the uredo host list. Trotter (1908), and Migula (1910, 1917), both include L. corniculatus and V. tenuifolia as hosts but it would appear that they have followed Fischer (1904). L. hispida and L. angustissimus were given as new hosts by Unanumo (1922), and Guyot (1939), respectively.

The rust is not very common in Britain although it is widespread. The first record for this country was on L. corniculatus (Grove 1911), and this is the most common host. According to Wilson (1934), the only record up to that year

in Scotland was from Berwick, but in 1949, Macdonald recorded it for both the St. Andrews and Inverness districts, on L. corniculatus. In England U. loti has been recorded for numerous districts, Mayfield (1935-7), Cooke G.J. (1935), Peel (1936), Brambley et al (1945), Mason & Grainger (1937). All these records are on L. corniculatus. Smith & Ramsbottom (1912-13), record the rust on Trifolium minus (= dubium Sibth) but this is probably in error for U. jaapianus Kleb. In Ireland the rust has been found on L. corniculatus (O'Connor 1936). All British authors agree that the aecidial stage is unknown in Britain (Wilson & Bisby 1954).

(ii) Host list of U. loti.

In the following host list, V. tenuifolia is included, but it is doubtful if this species can act as a host for U. loti. British species are marked thus!

Lotus corniculatus L. !	Wilson & Bisby (1954), Kirchner (1856), Saccardo (1888, 1912), Jordi (1904), Fischer (1904), Bubak (1908), Trotter (1908), Sydow (1904-12), Migula (1910, 1917), Grove (1911, 1913), Constantineanu (1920), Petrescu (1926), Guyot (1946, 1951), Macdonald (1949 a & b), Wilson (1934).
tenuifolius Presl.	Wilson & Bisby (1954), Bubàk (1908) Sydow (1904-12), Petrescu (1920), Saccardo (1912).

Lotus uliginosus	Schk.	Wilson & Bisby (1954), Saccardo (1912).
hispidis	Desf.	Wilson & Bisby (1954), Unamuno (1922), Guyot (1951).
tenuis	W. & K.	Guyot (1939).
angustissimus	L.	Wilson & Bisby (1954), Guyot (1939)
Vicia tenuifolia	Roth.	Fischer (1904), Trotter (1908), Migula (1910, 1917).
Euphorbia cyparissias	L.	Jordi (1904), Fischer (1904), Bubàk (1908), Sydow (1904-12), Constantineanu (1920), Guyot (1946)

From the above summary of the literature it is obvious that the major uredo host is L. corniculatus and that E. cyparissias is the only aecidial host.

(iii) Determination of uredo host range of U. loti at St. Andrews.

L. corniculatus is fairly common at St. Andrews. The rust U. loti, however, is uncommon and one of the local records (Macdonald 1949) is for the sand dunes where it is found amongst U. punctatus on A. danicus. The following experiment was carried out to determine if these two species of rust could change hosts.

Experiment 25.

Using the bell jar method the following inoculations were carried out:

U. punctatus on A. danicus was inoculated on to L. corniculatus and gave a - ve result.

U. punctatus on A. danicus was inoculated on to A. danicus and gave a + ve result.

U. loti on L. corniculatus was inoculated on to A. danicus and gave a - ve result.

U. loti on L. corniculatus was inoculated on to L. corniculatus and gave a + ve result.

The experiment was carried out in the greenhouse in September 1950 and again in January 1951. From the results it is apparent that U. punctatus and U. loti are different in their host reactions.

Experiment 26.

In Aug. 1953 the following experiment was carried out.

U. loti on L. corniculatus was collected from the sand dunes locality and inoculum was prepared by making a spore suspension. The method of inoculation and incubation of Experiment 1. was followed. The experimental plants were grown from seed obtained from various Botanic Gardens, or were wild plants dug up and potted in the greenhouse. The plants were inoculated on 5/7/53 and examined on 20/7/53. Incubation was for 2 days. 2 pots of each species of plants were inoculated and one was kept as a control.

The following plants were inoculated:-

L. corniculatus L.	Medicago lupulina L.
L. uliginosus Schk.	M. hispida Gaert.
L. hispidis Desf.	M. falcata L.
Anthyllis vulneraria L.	M. arabica (L) All.
Trigonella foenum-graecum L.	Lathyrus pratensis L.
Onobrychis viciifolia Scop.	Sarothamnus scoparius (L) Wimm.

On examination (20/7/53) only Lotus corniculatus bore uredo sori. All the controls remained healthy.

Experiment 27.

Experiment 26 was repeated on 28/7/53 and examined on 11/8/53. The same results were obtained.

It/

It would appear from these experiments that U. loti at St. Andrews is narrowly specialised on L. corniculatus.

(iv) Synonyms of U. loti.

The following are the synonyms of U. loti.

Puccinia loti Kirch. Kirchner (1856), Saccardo (1888 1912).
Uromyces euphorbiae-corniculati Jordi Jordi (1904), Fischer (1904), Bubák (1908), Trotter (1908).
U. striatus loti (Blytt) Arth. Arthur (1934).

(d) Uromyces striatus Schroet.

(i) Literature review. This species was first described by Schroeter (1869). Only uredo and teleutospores were found and the hosts given were: Genista tinctoria, Medicago sativa, M. falcata, M. lupulina, M. scutellata and Trifolium arvense. In 1875 he extended the host list to include Lotus corniculatus and Ervum lens (=Vicia hirsuta (L) Gray?). Later, in 1884, he inoculated T. aureum with aecidia from Euphorbia cyparissias and obtained uredo infections which agreed with U. striatus. He thus established the heteroecious nature of the rust. According to this paper, the aecidia deformed E. cyparissias in a similar way as U. pisi, but the leaves were shorter and broader and the plant was generally smaller. For hosts, he states that the rust occurs on legumes especially Trifolium and gives 3 species of Lotus, 3 species of Trifolium and 7 species of Medicago. In the form of the rust on the Medicago species, the/

the teleutospores tend to resemble the large punctations of U. genistae Schroet. rather than the striate condition of the forms on the other genera.

Fischer (1904) describes the species fully and gives a comprehensive host list. This included 9 species of Medicago, 8 species of Trifolium, 1 species of Lotus and a few others. He adds that it is questionable if all the forms of rust on these hosts are identical with U. striatus.

In the same year (1904) Klebahn, discussing Schroeter's (1884) work questions whether any difference exists between the deformation of E. cyparissias caused by U. pisi and U. striatus His host list includes those given by Schroeter (1889) and he adds 3 new species of Trifolium. He considers that the host lists given by Schroeter (1884, 1889) require confirmation.

Bubák (1908) records U. striatus Schroet. as a synonym for U. medicaginis falcatae (D.C.) Wint. No new host plants were added.

Cultures of various leguminous rusts were carried out by Treboux (1912) over a period of years. With aecidia upon E. virgata he inoculated numerous Astragalus species along wit Caragana arborescens and M. falcata. Only the Astragalus species were infected. Uredospores from these plants did not infect species of Medicago, Trifolium, Lotus, Caragana and Mellilotus. In another experiment aecidiospores from E.virgat and/

and E. gerardiana gave a strong infection on C. frutescens and uredospores of the latter could infect C. arborescens but not species of Medicago, Trifolium, and Mellilotus. In yet another experiment aecidiospores from the same source infected Medicago falcata and M. lupulina but were negative on Caragana, Lotus and Mellilotus species. The uredospores on Medicago lupulina could infect M. falcata and M. sativa, but not species of Lotus, Astragalus, Caragana or Mellilotus. The rust on Medicago falcata produced infection on M. lupulina, M. sativa, M. scutellata, M. ciliarus, M. echinus, M. trunculata, and M. hispida.

The work of Treboux has been discussed in some detail as it serves to indicate the difficulties encountered in culture work with this group of rusts. Obviously, the aecidiospores on E. virgata used by Treboux were a mixed culture.

Arthur (1934) describes U. striatus as a compound specie and gives 2 'new combinations': U. striatus medicaginis (Pass) Arth. and U. striatus loti (Blytt) Arth. It is the former combination which is synonymous with U. striatus Schroet.

Koepper (1942) carried out some pathogenicity tests to determine the resistance of alfalfa species to U. striatus. Those which were susceptible included the Russian species M. ruthenica.

Guyot (1951) using aecidiospores from E. cyparissias inoculated a number of species of several genera of legumes. Positive/

Positive results were obtained only in the case of Medicago species and the author concluded that this represented one race of U. striatus.

As is well known to British uredinologists, the fungus on T. minus referred to by Grove (1913), as U. striatus, is now considered to be U. jaapianus Kleb. (Ramsbottom 1914, Grove & Chesters 1933). Grove (loc.cit.) had noted the difference between the specimen he described and the description figured by Fischer (1904), and suggested it may not be the same rust. The rust U. striatus has been recorded for several districts in Britain; Macdonald (1939, 1949), Glasscock & Ware (1946), Ainsworth (1953), Gregory (1951), Brambley (1943), Hadden (1916 Grove & Chesters (1933), Brooks (1953), Musket et al (1931-32), Wilson & Bisby (1954), Sampson & Western (1941), Smith & Ramsbottom (1912-13). According to Wilson & Bisby (loc.cit.) Hadden's (1916) report is in error for U. jaapianus Kleb.

Glasscock & Ware (loc.cit.) suggest that the only British host species is M. arabica as all specimens of U.striat on Trifolium species have been found on examination to be U. jaapianus. These authors claim a first record for Britain of U. striatus on M. lupulina but were apparently unaware that this species had already been recorded as a host for U. striatu in Scotland (Macdonald 1939).

At/

At St. Andrews the rust is rare and occurs only on Medicago lupulina (Macdonald 1939, 1949). The first known British specimen was collected by Dr. M. Wilson in 1903 (Wilson & Bisby, loc.cit.). Of the 17 specimens of U. striatu examined by the writer, 14 were on species of Medicago, 2 on Trifolium campestre, and 1 on T. arvense. As can be seen from Tables 18 and 24 there is no significant difference in spore size between the specimens on Trifolium and Medicago. Most of these specimens were from the Continent or the U.S.A. It appears, however, that the species of rust on Trifolium and labelled U. striatus in Britain, require confirmation (Glasscock & Ware, 1946).

The following list gives the host plants recorded in the literature for U. striatus. British host species are recorded thus !.

(ii) Host list of U. striatus.

Genista tinctoria L.	Schroeter (1869).
Medicago sativa L.!	Schroeter (1869, 1875, 1889), Passerini (1877), Saccardo (1882), Roumeguère (1892), Fischer (1904), Bubák (1908), Trotter (1908), Treboux (1912), Arthur (1912, 1934), Constantineanu (1920), Savulescu (1941), Koepper (1942), Guyot (1951), Sampson & Western (1941)
falcata L. !	Schroeter (1869), Passerini (1877), Fischer (1904), Bubák (1908), Trotter (1908), Treboux (1912), Arthur (1912, 1934), Constantineanu (1920), Savulescu (1941), Koepper (1942).
ruthenica Trautv.	Koepper (1942).
lupulina/	

Medicago lupulina L. !	Schroeter (1869,1889), Fischer (1904), Trotter (1908), Treboux (1912), Arthur (1912,1934), Constantineanu (1920), Savulescu (1941), Macdonald (1939,1949 Brooks (1953), O'Connor (1936), Gregor (1951), Sampson & Western (1941), Wilso & Bisby (1954), Glasscock & Ware (1946)
scutellata Mill.	Schroeter (1869), Fischer (1904), Treboux (1912).
minima L. !	Schroeter (1875,1889), Fischer (1904), Bubàk (1908), Treboux (1912), Constantineanu (1920), Savulescu (1941 Guyot (1938, 1951).
littoralis Rhod.	Schroeter (1889), Fischer (1904).
hispida Gaertn. !	Dietel & Neger (1897), Spegazzini (1902 Trotter (1908), Arthur (1912,1934), Guyot (1951), Grove & Chesters (1933) Wilson & Bisby (1954), Fischer (1904).
orbicularis All.	Fischer (1904), Trotter (1908), Guyot (1951).
turbinata Willd.	Fischer (1904).
disciformis D.C.	Trotter (1908).
arabica (L) All. !	Trotter (1908), Guyot (1950), Brambley (1943), Ainsworth (1953), Wilson & Bisby (1954), Sampson & Western (1941) Robertson (1953),
muricata All.	Trotter (1908).
ciliarius Krock.	Treboux (1912).
echinus D.C.	Treboux (1912).
trunculata Gaertn.	Guyot (1951), Treboux (1912).
Trifolium arvense L. !	Schroeter (1869, 1889), Passerini (187 Fischer (1904), Bubàk (1908), Trotter (1908), Treboux (1912), Constantineanu (1920), Savulescu (1941).
aureum Poll. !	Schroeter (1875,1889), Fischer (1904), Bubàk (1908), Constantineanu (1920)
dubium Sibth. !	Schroeter (1889), Fischer (1904) Smith & Ramsbottom (1912-13), Musket et al (1931-2).
micranthum Viv. !	Fischer (1904), Bubàk (1908).
striatum L.	Fischer (1904).
caroliniamus Michx.	Fischer (1904), Klebahn (1904).
campestre Schreb. !	Trotter (1908), Savulescu (1941), Guyo (1938), Fischer (1904), Hadden (1916).
Lotus corniculatus L. !	Schroeter (1875,1889), Trotter (1906).
tenuifolius Presl.	Schroeter (1889).
uliginosus Schnk. !	Schroeter (1889), Fischer (1904).
edulis L.	Trotter (1908).
Euphorbia cyparissias L. !	Schroeter (1884), Fischer (1904), Klebahn (1904), Sydow (1904-12), Bubàk (1908), Trotter (1908), Hariot (1908), Migula (1910,1917), Arthur (1934), Guyot (1951).

virgata/

virgata W & K. !	Treboux (1912).
gerardiana Jacq.	Treboux (1912).

The rust is referred to as U. striatus Schroet. by the following authors:- Fischer (1904), Sydow (1904-12), Saccardo (1882, 1888), Savulescu (1941-2), Klebahn (1892,1904), Roumeguère (1897), Hariot (1892,1908), Schroeter (1869,1870,1871,1875, 1889), Briosi & Cavara (1891), Dietel (1891), Treboux (1912), Saccardo & Trotter (1913), Passerini (1877) Koepper (1942), Trotter (1908), Guyot (1938,1946,1951), Migula (1910,1917), Constantineanu (1920), for the Continent, and Brooks (1953), Glasscock & Ware (1946), Musket et al (1931-2), Sampson & Western (1954), Macdonald (1939,1949), Grove & Chesters (1933) Grove (1911), Smith & Ramsbottom (1912-13), Plowright (1889), Gregory (1951), Brambley (1943), Hadden (1916), O'Connor (1936 and Wilson & Bisby (1954), for Britain.

(iii) Synonyms of U. striatus.

Uredo fabae Pers	Greville (1824) p.p., Oudemans (1921).
U. medicaginis Speg.	Spegazzini (1902), Saccardo (1905)?
U. anthyllidis Grev.	Greville (1824), Oudemans (1921).
Uromyces trifolii Fuck.	Saccardo (1875), Fuckel (1869-70), Oudemans (1921).
U. medicaginis Pass.	Saccardo (1875), Passerini (1877), Oudemans (1921).
U. medicaginis Sacc.	Saccardo (1875), Bubàk (1908), Trotter (1908).
U. medicaginis falcatae (DC) Wint.	Bubàk (1908), Trotter (1908)
U. m. falcatae Wint.	Roumeguère (1897), Oudemans (1921), Winter (1884).
U. leguminosarum f. medicaginis Pass.	Bubàk (1908), Trotter (1908).
U. striatus medicaginis (Pass) Arth.	Arthur (1934).
U. minor Schroet ?	Dietel & Neger (1897), Savulescu (1941-2).

(e)/

(e) Uromyces jordianus Bubàk.

(i) Literature review. Winter, in his Kryptogamen Flora (1884), united under Uromyces Genistae-tinctoriae (Pers) Wint. all the leguminous rusts whose aecidial stages were unknown at that time. Jordi, (1903,1904) whose work has already been referred to, separated this composite rust into several species and included in these was a species which was narrowly specialised on Astragalus exscapus. He named this species U. astragali (Opiz) Jordi, emend. The aecidial stage was unknown.

Fischer (1904) citing Jordi's work and conclusions adds that the rust probably belongs to the heteroecious Uromyces group.

Bubàk (1905) pointed out that Jordi's (1903,1904) naming of this species was not in accordance with the rules of priority, and consequently he proposed the new name of U. jordianus Bubàk n.s. It should perhaps be noted here that in 1907, Magnus named a rust on Vicia cracca as U. jordianus Magn. but later that year in view of Bubàk's new name for Jordi's U. astragali (Opiz) Jordi, changed it to U. fischeri-eduardii. In all later works the rust has been referred to as U. jordianus Bubàk (nec Magnus).

Up to the publication of Fischer's paper in 1923, this species was considered to have no aecidia. Fischer inoculated Euphorbia/

Euphorbia cyparissias and E. gerardiana with teleutospores of U. jordianus. After 3 years (autumn 1920 - spring 1923), pycnidia appeared on E. cyparissias but the leaves fell off before aecidia could be formed. Fischer states himself that the heteroeciousism of this species is not conclusively proved but adds that E. cyparissias is the probable host.

Guyot (1951), however, states that the rust is heteroecious though he does not mention the aecidial host.

A. exscapus is not native in Britain and the rust has never been found here.

(ii) Host list of U. jordianus.

As noted above A. exscapus is the only uredo host and this has been quoted by Saccardo (1912), Jordi (1904), Fischer (1904,1923), Bubák (1905,1908), Migula (1910,1917), Sydow (1904-12), Kobel (1921) and Guyot (1951).

Fischer's (1923), is the only reference to the aecidial stage.

(iii) Synonym of U. jordianus.

The only synonym for the rust is U. astragali (Opiz) Jordi emend. quoted by Jordi (1904) and Fischer (1904).

(f)/

(f) Uromyces fischeri-eduardii Magn.

(i) Literature review. One of the specialised forms of U. pisi which Jordi (1903, 1904) discovered, was confined to Vicia cracca in the uredo stage. This variety was given specific rank by Magnus (1907 p.252) although he gave no description of the rust. He named it U. jordianus Magn., but later after Bubàk's correction (1905) of Jordi's (1904) nomenclature (vide section on U. jordianus Bubàk) he changed the name to U. fischeri eduardii Magn.

Jordi (1904) had already shown that it was heteroecious and E. cyparissias was the aecidial host. Most of the rust floras consulted by the writer give this aecidial host and V. cracca as the only uredo host.

Mayor (1932) after a series of experiments concluded that the aecidial stage occurred only on E. cyparissias. Later however, (1939) he obtained aecidial infections on E. cyparissias and E. virgata after inoculating with teleutospores on V. cracca. The reverse inoculations gave positive results with V. cracca and V. sativa, and on this basis he suggested the possibility of races. Aecidia on the hybrid E. cyparissia x virgata gave positive infections on V. cracca.

In the same year Guyot (1939) carried out a series of inoculation experiments and obtained infections only on E. cyparissias and V. cracca. Negative results were obtained with/

with V. sepium, V. sativa, Lathyrus species and other legumes.

Vicia cracca is fairly common in Britain but the rust has not yet been recorded.

(ii) Host list of U. fischeri eduardii.

The following list gives the hosts recorded in the literature:-

V. cracca. L.	Bubàk (1908), Magnus (1907), Sydow (1904-12), Saccardo (1912), Savulescu (1920), Mayor (1939), Migula (1910, 1917), Guyot (1938-9), Constantineanu (1920).
U. sativa L.	Mayor (1939).
E. cyparissias L.	Bubàk (1908), Sydow (1904-12), Saccardo (1912), Mayor (1932,1939), Guyot (1939), Migula (1910, 1917), Constantineanu (1920).
E. virgata W.&K.	Mayor (1939).

(iii) Synonym of U. fischeri eduardii.

The only synonym is U. jordianus Magn (nec Bubàk).

(g) Uromyces laburni (D.C.) Fuckel.

(i) Literature review. Persoon (1801) referred to this species as Uredo appendiculata ϒ genistae tinctoriae. Four years later (1805), De Candolle gave it the name Puccinia Laburni. The rust was described as species of Cytisus and Genista. Strauss (1810),gave separate names to the rusts on each of these host genera as Uredo cytisi and U. genistae respectively. Fuckel (1860) records two forms of rust which obviously belong here: Uromyces apiculatus Lèv f. laburni Fuck and U. leguminosarum f. genistae Fuck. Ten years later he apparently combines these forms under one species: U. genistae Fuck.

Schroeter/

Schroeter (1875), was the first to give a comprehensive host list including 6 species of Cytisus and 3 of Genista. He placed it in his Hemi-uromyces group, but suggested that aecidia possibly exist. Five years later, Winter (1880) proposed that all the Hemi-uromyces on leguminous hosts should be grouped together under the name U. genistae tinctoriae (Pers Wint. and in 1884 he carried out this proposal. The species of rusts which he included under this composite name included such present day species as U. striatus, U. punctatus and U. loti. The host list given by Winter (loc.cit.) is consequently very large and is not repeated here. Hariot (1892), citing Winter (1884), points out that some of the forms included under U. genistae tinctoriae can be separated on morphological and biological bases, e.g. U. punctatus. Subsequent to Hariot's paper the rust U. genistae tinctoriae (Pers) Wint. became strictly synonymous with U. genistae Fuck.

Arnhart (1883) reported finding aecidia of U. genistae tinctoriae on species of Cytisus, but his determination of the rust is doubtful.

The heteroecious nature of the rust was demonstrated by Treboux (1912). He inoculated Caragana frutescens with aecidia from E. virgata and E. gerardiana and obtained strong infections. The rust on C. frutescens could infect C. arborescens. Species of Genista and Cytisus were not at his disposal. Dietel (1919, 1922), confirmed Treboux's work and added E. cyparissias to the aecidial host list.

The/

The rust is not recorded by Grove (1913), and Moore (1933-42), states that the first record for England was in 193
In Scotland the species was first found by Macdonald in 1934 on Genista anglica, and Wilson (1934) claims this as a first record for Britain. Moore (loc.cit.), although giving the locality where the rust was found in 1933, does not quote any published record of it. Macdonald (1946), carried out a series of cross inoculations with this species and concluded that 3 specialised forms existed as forma anglicae on G. anglica; forma scoparii on Sarothamnus scoparius; and forma ulicis on Ulex europeaus. The rust has been reported elsewhere in Britain: Mayfield (1935-7) on S. scoparius and G. tinctoria; Brambley et al (1945) on Laburnum anagyroides a new host for Britain; Grove (1934-35) on G. sagittalis; and Wilson (loc.cit. on G. tinctoria. Wilson & Bisby (1954) give all the above host

The aecidial stage has never been found in Britain nor has it been demonstrated experimentally in the greenhouse (vide e.g. Macdonald 1946).

(ii) Host list of U. laburni.

The host list for U. laburni is given below. The species of Cytisus is recorded below under the genus Sarothamnu

Genista tinctoria L.	Hariot (1892), Strauss (1810), Saccard (1873), Kobel (1921), Bubák (1908), Schroeter (1875,1889), Guyot (1939), Dupais (1946), Trotter (1908), Constantineanu (1920), Winter (1880, 1884), Sydow (1904-12), Moore (1933-42 Mayfield (1935-7), Wilson (1934), Wilson & Bisby (1954).

sagittalis/

sagittalis L. Strauss (1810), Guyot (1946), Fuckel (1860,1869-70), Constantineanu (1920), Schroeter (1875), Oudemans (1921), Grove (1934-5).

hispanica L. Guyot (1939).

sessilifolia D.C. Guyot (1939), Oudemans (1921), Winter (1880

sericea Wulf. Guyot (1946), Oudemans (1921), Saccardo (187

germanica L. Schroeter (1875,1889), Dupais (1946), Trotter (1908), Oudemans (1921).

anglica L. ! Schroeter (1875), Winter (1880), Saccardo (1888), Sydow (1904-12), Moore (1933-42 Macdonald (1946), Wilson (1934), Wilson & Bisby (1954).

Sarothamnus scoparius (L.) Wimm. ! Schroeter (1889), Macdonald (1946), Mayfield (1935-7), Wilson & Bisby (1954).

Cytisus alpinus Mill. Schroeter (1875), Oudemans (1921), Saccardo (1888), Sydow (1904-12).

falcatus W.& K. Savulescu (1941-2), Oudemans (1921), Saccardo (1888), Sydow (1904-12), Fuckel (1860).

sessilifolius L. Hariot (1892), Guyot (1946).

leuchotriches ? Savulescu (1941-2).

hirsutus L. Hariot (1892), Savulescu (1941-2), Schroeter (1875), Winter (1880), Saccardo (1888), Sydow (1904-12), Oudemans (1921).

heuffeli Wierzh. Oudemans (1921), Constantineanu (1920), Winter (1884), Saccardo (1888), Sydow (1904-12).

biflorus l'Herit. Bubàk (1908), Schroeter (1875), Oudemans (1921), Winter (1880), Saccardo (1888), Sydow (1904-12).

prolifer Kit. Oudemans (1921), Sydow (1904-12).

capitatus Scop. Bubàk (1908), Schroeter (1889), Oudemans (1921)

nigricans L. Bubàk (1908), Schroeter (1875,1889), Strauss (1810), Constantineanu (1920), Oudemans (1921), Saccardo (1888), Sydow (1904-12).

ratisbonensis Schiff. Schroeter (1889), Oudemans (1921), Winter (1880).

supinus L. Winter (1880), Oudemans (1921).

Caragana frutescens Med. Treboux (1912), Savulescu (1941-2), Constantineanu (1920), Oudemans (1921).

arborescens Lam. Treboux (1912), Savulescu (1941-2), Winter (1884), Sydow (1904-12).

Colutea arborescens L. ! Schroeter (1889), Constantineanu (1920), Winter (1880).

Galega officinalis L. ! Bubàk (1908), Schroeter (1898), Winter (1880).

Lotus/

Lotus angustissimus L.	Guyot (1946).
Ulex europeaus L. !	Macdonald (1946), Wilson & Bisby (195
Laburnum anagyroides Medic. !	Brambley et al (1954), Wilson & Bisby (1954), Hariot (1892), Bubák (1908), Schroeter (1875,1889), Savulescu (1941-2), Strauss (1810), Guyot (1939), Fuckel (1860), Oudemans (1921), Saccardo (1888), Sydow (1904-12).
Euphorbia virgata W.& K.	Treboux (1912).
gerardiana Jacq.	Treboux (1912).
cyparissias L. !	Dietel (1919, 1922).

(iii) Synonyms of U. laburni.

The synonyms of U. laburni are given below.

Uredo appendiculata V. genistae tinctoriae Pers:	Persoon (1801), Saccardo (1888), Strauss (1810
U. cytisi D.C.	Schroeter (1875), Oudemans (1921).
U. laburni D.C.	D.C. (1805), Saccardo (1888).
U. cytisi Strauss.	Saccardo (1888), Strauss (1810).
U. leguminosarum var. genistarum Rabenh.	Saccardo (1888).
U. genistae Strauss.	Strauss (1810).
Aecidium euphorbiae Gmel.	Dietel (1919).
Puccinia laburni D.C.	D.C. (1805), Saccardo (1888).
Uromyces laburni Fuckel.	Fuckel (1860), Saccardo (1888), Schroeter (1875), Oudemans (1921).
U. cytisi Schroet.	Saccardo (1888).
U. onobrychidis Lèv.	Saccardo (1888).
U. genistae-tinctoriae (Pers.) Fuck.	Saccardo (1888), Trotter (1908).
U.g. tinctoriae (Pers.) D.C.	Hariot (1892).
U.g. tinctoriae (Pers.) Wint.	Treboux (1912,1912a), Bubák (1908), Winter (1884), Sydow (1904-12 Savulescu (1941-2), Guyot (1939,1946 Dupais (1946), Constantineanu (1920), Migula (1910, 1917).
U.g. tinctoriae (Pers.).	Schroeter (1889).
U.genistae (Str.) Fuck.	Saccardo (1823).
U.genistae Schroet.	Fischer (1904).
U.genistae Fuck.	Fuckel (1869-70), Schroeter (1875), Oudemans (1921).
U.g. tinctoriae Wint.	Moore (1933-42).
U. laburni (D.C. Schroet.	Schroeter (1875).
U. oxytropidis Kze.	Schroeter (1875).
U. apiculatus Lèv. f. laburni Fuck.	Fuckel (1860).
U. leguminosarum f. genistae Fuck.	Fuckel (1860).
U. laburni (D.C.) Fuck.	Savulescu (1941-2), Wilson & Bisby (1954).

Table 31.

U. pisi (Pers. ex D.C.) Fuck.	U. lathyri-latifolii Guyot.
(a) Uredospores	(a) Uredospores.
21-24 (19-28) x 19-22 (16-23) u.	24-27 (21-32) x 21-23 (19-27).
Epispore 1.5 to 2 u thick. Rarely 2.5 u.	Epispore 2-2.5 u thick.
Germ pores 3-5. Mostly 3-4.	Germ pores 3-6. Mostly 4-5.
(b) Teleutospores.	(b) Teleutospores.
'Form' somewhat irregular.	'Form' very irregular.
22-27 (20-30) x 18-21 (16-23).	24-31 (21-42) x 16-19 (15-27).
Epispore distinctly (often densely) punctate - verrucose on the whole surface, without any appearance of being arranged in linear order.	Epispore with indistinct superficial ornamentation, appearing almost smooth, or consisting of very small warts disposed at random or more often in parallel longitudinal or oblique lines.

Table 31. Table comparing the morphology of U. pisi and U. lathyri-latifolii.

(h) Other species.

Apart from the species of the U. pisi group just discussed, there are 7 other species of rust which appear to be closely related and probably belong in this group. In all these species, the uredo stage occurs on various legumes and where aecidia have been found, these occur on Euphorbia species Notes on these 7 species are given below.

1. Uromyces lathyri-latifolii Guyot.

This species was first described by Guyot in 1939. As the specific epithet suggests, the main uredo host of the rust is Lathyrus latifolius. The only other host which has been recorded for this rust as far as the writer can determine, is the variety L. latifolius angustifolius (Dupais 1946.) It thus appears that the uredo stage is narrowly specialised on these two hosts.

According to Guyot (1939), it is closely allied to U. pisi morphologically. In general the spores of U. l. latifolii are slightly larger than those of U. pisi and this is brought out in the table of comparison (Table 31).

The above descriptions are taken from Guyot (loc.cit.). As can be seen from the table the main differences between U.l. latifolii and U. pisi are the slightly larger spores, and the thicker uredospore walls of the former. It is doubtful if the differences in the sculpturing of the teleutospore wall will be of/

Table 32.

U. fischeri eduardii Magn.	U verrucosae-craccae Mayor.
(a) Uredospores 20-26 µ diam. Wall 1.5-2 µ thick Germ pores 3-5. (b) Teleutospores 24-30 x 19-25 µ . Wall 1.5-2 µ. Densely and minutely verrucose.	(a) Uredospores. 18-23 µ diam. Wall 2-2.5 µ thick. Germ pores 5-6. (b) Teleutospores. 19-23(18-28) x 18-20(16-21) µ . Wall 2-2.5 µ. Densely and minutely verrucose. Apical papilla up to 3 u. thick.

Table 32. Table comparing the morphology of U. fischeri eduardii and U. verrucosae-craccae.

of much assistance in identifying this species, for it has been shown that even within U. pisi, the sculpturing varies (figs. 19-26, Plates IV and V).

L. latifolius is very rare in Britain and is native to south and central Europe.

The rust has not been found in Britain.

2. U. verrucosae-craccae Mayor.

This species was originally described by Mayor (1939) after a series of culture experiments and observations carried out over a number of years. He concluded that the rust was heteroecious, that its aecidial host was Euphorbia verrucosa, and that Vicia cracca was the uredo host. Experiment showed that although U. fischeri eduardii (which is similar morphologically and has the same uredo host), could produce aecidia on E. cyparissias and not on E. verrucosa, U. ver. craccae could infect E. verrucosa but not E.cyparissias On the basis of this and slight morphological differences he distinguished his species by giving it specific rank. The following table (32) compares U. f. eduardii, according to Fischer (1904) with U. ver craccae according to Mayor (loc.cit

As can be seen from the table, the sculpturing of the teleutospore walls is similar, but the walls of both the uredospores and teleutospores of U. ver craccae, are thicker than those of U. f. eduardii. Mayor (loc.cit.) in discussing these/

these two rusts, states that the number of germ pores for U. f. eduardii is 5-7, but the writer has never found this number given in the literature including the original description (Magnus 1907). From the table it is seen that the number of germ pores of U. ver. craccae is greater than U. f. eduardii, and the measurements of the uredospores and teleutospores are smaller.

This rust has not been found in Britain.

3. U. viciae craccae Const.

This species was first described by Constantineanu (1904) who gives the following description:- Uredospores, 19-24 x 18-25.5 μ with an epispore 2-2.5 μ thick and 5 germ pores. The teleutospores measure 18.5-23 to 21.6-27 x 20.7-22.2 μ with a pedicel up to 40 μ long.

The hosts given are Vicia cracca, Lens esculanta, and V. tenuifolia, and he places it in his Micro (=lepto) Uromyces as only teleutospores were present. Paraphyses were found in the sori. He notes that the sculpturing on the teleutospore walls consisted of longitudinal striations and that because of this it resembled U. striatus.

Magnus (1890), reported a rust on V. tenuifolia and placed it under U. striatus. No paraphyses was present. The same specimen was examined by Fischer (1904) who provisionally placed it with U. loti. According to Magnus (1907) the/

the sculpturing on the teleutospore wall and the smaller dimensions of this species distinguish it from U. striatus and consequently he placed it under U. viciae craccae. He also remarks (loc.cit.) that although Constantineanu (1904) described only teleutospores and the presence of paraphyses, the lack of uredospores was probably due to the time of collection of the rust (September) and the paraphyses were probably the pedicels of matured uredospores.

The aecidia have not been reported in the literature, though Bubàk (1908) suggests that E. cyparissias may be the aecidial host.

The rust has not been found in Britain.

The host list as determined from the literature is given below.

Vicia cracca L.	Migula (1910,1917), Constantineanu (1904, 1920), Savulescu (1941-2), Guyot (1946), Magnus (1907).
V. tenuifolia Roch.	Migula (1910, 1917), Magnus (1907), Constantineanu(1904, 1920).
Lens esculanta Moen.	Migula (1910, 1917), Bubàk (1908), Constantineanu(1904, 1920), Magnus,(1907).

4. U. klebahni Fisch.

This species was described by Fischer in 1914. The original paper was unobtainable by the writer, and the notes given below have been compiled from Kobel (1921) and Guyot (1951).

In/

In 1921, Kobel demonstrated that the rust was heteroecious and that the aecidial host was E. cyparissias. He inoculated Astragalus monspessulanus and A. onobrychis with aecidia from E. cyparissias and obtained uredo infections. The rusts on these two Astragalus plants were identical morphologically, but they produced different deformations on their respective hosts. Only the form on A. monspessulanus could infect A. alpinus. Kobel also examined a number of herbarium specimens of U. klebahni and concluded that several different 'forms' of the rust existed. His conclusions were arrived at by comparing the spore measurements of these specimens, but he gives no figures nor does he describe his methods.

The following description of U. klebahni is taken from Guyot (1951):- The uredospores measured 24-27(22-29) x 21-23(18-25) µ with 3-5 germ pores and a wall thickness of 2.5 µ. The teleutospores measured 22-25(17-27) x 16-19(15-22) µ. The wall is 1-1.5 µ thick with small rather dense warts.

The hosts given by Kobel (1921) are: A. onobrychis, A. monspessulamus, A. alpinus, A. leontinus, A. glycyphyllos, A. sempervirens, A. menziensis, Oxytropis halleri and E. cyparissias. Out of this list only the first three and E. cyparissias were determined experimentally. Guyot (1951) gives only A. monspessulanus.

5./

5. U. jaapianus Kleb.

This species was originally described by Klebahn (1913 on Trifolium species. The uredospores have thick walls (2-3 μ) with distinct spines 2.5-3 μ apart. The spores measure 20-25 x 20-23 μ and have 5-6 germ pores. The teleutospore walls are of the same thickness as the uredospores and have thicker spines 2.5-3 μ apart. These spores measure 19-22 x 16-20 μ.

In Britain (and no doubt on the continent too) this species has been confused with U. striatus, e.g. Smith & Ramsbottom (1912-13)?, Grove (1911, 1913), Hadden (1916,1920) and Musket et.al. (1931-2). In all these records the rust is referred to as U. striatus and the host given is T. dubium Sibth.

Under U. jaapianus the rust is recorded in Britain by: Grove & Chesters (1933), Smith & Ramsbottom (1913), Wilson (1934), Mayfield (1935-7), Sampson & Western (1954), Brooks (1953), Glasscock & Ware (1946), Ramsbottom (1914) and Wilson & Bisby (1954). The hosts given by these authors are T. dubium and T. campestre. Klebahn (1913) gives only T.dubiu

An accidial host has not been recorded for this rust.

In Britain the rust is rare but has been found as far north as Aberdeen (Wilson 1934) and also in the south of England, e.g. Mayfield (1935-7).

6./

Tables 33 and 34.

Author	Diameter	G.P.	W.
Plowright (1889)	22-24 μ	4-5	-
Schroeter (1875,1889)	22-24	4-5	3.5
Bubák (1908)	20-25	4-5	3.5
Fischer (1904)	20-25	5-8	3.5
Grove (1913).	18-25	4-6	3-3.5

Table 33. Showing uredospore measurements (Diam), number of germ pores (G.P.) and wall thickness (W) of U. anthyllidis.

Author	L.	B.
Plowright (1889)	19-22	17-20
Schroeter (1875,1889)	19-22	17-20
Bubák (1908)	18-21 diam.	-
Fischer (1904)	22-28	17-19
Grove (1913)	16-22	15-20

Table 34. Showing teleutospore measurements of U. anthyllidis. Figures in microns.

6. U. heimerlianus Magn.

This species was first described and figured by Magnus (1907). The uredospores measure 18-24 μ diam. with 3-5 germ pores and a wall thickness of 1.5 μ. The teleutospores measure 18-25 x 20-30μ and have a wall thickness of 1.5 μ.

The rust is not common on the Continent and is absent from Britain.

According to Saccardo (1912), the spores resemble U. fischeri-eduardii, but are slightly smaller.

The host list is:-

Vicia hirsuta L. !	Saccardo (1912), Magnus (1907), Savulescu (1941-2), Sydow (1904-12).
V. cassubica L.	Savulescu (1941-2).
V. pannonica L.	Savulescu (1941-2).

7. U. anthyllidis Schroet.

This species was first described by Greville (1824-26) under the same Uredo anthyllidis, which is the only synonym. The species belongs to the Hemi-uromyces group.

Tables 33 and 34 give the spore measurements as determined from the literature.

The following data on U. anthyllidis was obtained by the writer from local specimens. The uredospores measured 19-25 x 20-24 μ, with a mean of 23.2 x 21.8 μ. The wall thickness was 3.5 μ and the number of germ pores varied from 4-6. The teleutospores measured 18-22.5 x 17.5-20.5 μ, with a mean of 20.1 x 18.8 μ. The wall was 2.5 μ thick.

Jordi/

Jordi (1904) inoculated species of Lupinus, Trigonella and Ononis along with Anthyllis vulneraria and A. montana, with uredospores of U. anthyllidis. Only A. vulneraria became infected. The experiment was repeated twice with the same results. Fischer (1904), placed under U. anthyllidis a rust on Coronilla varia and one on L. alba. He suggested th the rust on Lupinus was a biological race and that this suggestion was supported by Jordi's experiments (1904).

In Britain the rust occurs only upon A. vulneraria: Grove (1913), Plowright (1889), Macdonald (1949), Wilson (1934), Adams & Pethybridge (1909-10), Mason & Grainger (1937 Sampson & Western (1941). At St. Andrews, uredospores are produced in abundance but few teleutospores have been seen.

The following is the host list:-

Anthyllis vulneraria. !	Schroeter (1875, 1889), Hariot (1892 Bubák (1908), Jordi (1904), Fischer (1904), Constantineanu (1920), Grove (1913), Plowright (1889), Wilson (1934), Macdonald (1949), Sampson & Western (1941), Mason & Grainger (1937), Adams & Pethybridge (1909-10
A. maritima.	Grove (1913) for the Continent.
Lupinus lutea.	Schroeter (1875, 1889).
L. angustifolia.	Schroeter (1875, 1889).
L. alba.	Fischer (1904)?
Coronilla varia. !	Constantineanu (1920), Fischer (1904

The only synonym is Uredo anthyllidis Grev.

Table 35.

Species	Heter.	Ured. stage recorded for		Aec. stage recorded for		Pluriv.		No. of Hosts.					
								Ured.			Aecid.		
		Cont.	Brit.	Cont.	Brit.	Cont.	Brit.	Cont.	Actual Brit.	Poss. Brit.	Cont.	Actual Brit.	Poss. Brit.
U. punctatus	+	+	+	+	-	+	-	42	1	6	3	-	2
U. pisi	+	+	+	+	+	+	+	24	2	11	7	1	4
U. loti	+	+	+	+	-	-	-	7	5	5	1	-	1
U. striatus	+	+	+	+	-	+	+	27	4	13	3	-	2
U. laburni	+	+	+	+	-	+	+	28	6	6	3	-	2
U. klebahni	+	+	-	+	-	+	-	8	-	3	1	-	1
U.ver.craccae	+	+	-	+	-	-	-	1	-	1	1	-	-
U.fisch.eduardii	+	+	-	+	-	-	-	2	-	2	2	-	2
U. jordianus	?	+	-	?	-	-	-	1	-	-	?	-	-
U.vic.craccae	-	+	-	-	-	+	-	3	-	1	-	-	-
U. jaapianus	-	+	+	-	-	-	-	1?	2	2	-	-	-
U.lath.latifolii	-	+	-	-	-	-	-	2	-	1	-	-	-
U. anthyllidis	-	+	+	-	-	+	-	6?	1	2	-	-	-
U. heimerlianus	-	+	-	-	-	-	-	1	-	1	-	-	-

Table 35. Showing the data on the host specialisation and geographical distribution of the U. pisi group.
Heter = Heteroecious; Cont. = Continent; Brit. = Britain; Pluriv. = Plurivorous; Ured. = Uredo; Aec. = Aecidial; Poss. = Possible;
+ = Yes; - = No.

V. Ecological and geographical data.

The data on the distribution and specialisation of the U. pisi group, presented in Section IV are summarised in Table 35.

Considering table 35, it will be seen that of the 14 members of the U. pisi group, only 7 are known in Britain, although the whole group is represented on the European continent. Eight of the group are heteroecious on the Continent, while only one (U. pisi) has been found in Britain with aecidia. Four of the heteroecious species, U. punctatus, U. striatus, U. loti and U. laburni are represented in Britain but only as Hemi-uromyces. On the Continent three of the eight heteroecious species have only one aecidial host, but each rust can form its aecidia on Euphorbia cyparissias, except U. verucossae craccae. U. pisi has seven aecidial hosts on the Continent but only one in Britain. A few of the aecidial hosts for all the heteroecious species on the Continent are found in Britain.

All the heteroecious rusts, excepting U. ver. craccae, U.f. eduardii, and U. loti, are plurivorous on the Continent, i.e. they have uredo hosts in more than one genus. Only three of the heteroecious rusts, U. striatus, U. pisi and U.laburni are plurivorous both in Britain and on the Continent. U. vic. craccae and U. anthyllidis are the only Hemi-forms which are also plurivorous.

Only/

Only six species of the whole group have seven or more uredo hosts. Five of these six species are represented in Britain, but in this country all have less than seven uredo hosts, i.e. U. punctatus, U. pisi, U. loti, U. striatus, and U. laburni. Considering the number of uredo hosts it will b seen from the table that the number of actual hosts for the British rusts is much smaller than the number of possible hosts. Exceptions are U. loti and U. laburni whose actual numbers of uredo hosts are the same as the possible numbers o uredo hosts. On a host basis, it is possible for the follow ing six rusts which are unknown in Britain to be represented in this country:- U. klebahni, U. ver. craccae, U. fischeri-eduardii, U. vic. craccae, U. lathyrus latifolii and U. heimerlianus.

From a consideration of the above data, the following points emerge.

1. Compared with the Continent, there is a reduction in th number of leguminous rusts in Britain. This is true for both Hemi- and Hetero- species.

2. The number of plurivorous rusts on legumes in Britain i also less than that on the Continent. The British species are also more specialised with regard to hosts, e.g. U. punctatus is recorded on numerous Astragalus species and o a few Oxytropis species on the Continent but occurs only on one species of Astragalus in Britain.

3./

3. Members of the group which are highly specialised on the Continent, e.g. U. lathyri latifolii and U. jordianus, do not occur in Britain. A possible exception is U. jaapianus which is only recorded for one species of Trifolium on the Continent and two in Britain, but as has already been suggested, probably a number of the continental records for U. striatus are in error for U. jaapianus. U. klebahni is also a possible exception, for although it is recorded for eight uredo hosts on the Continent, (Kobel 1921), only three of these have been determined experimentally.

4. All the Hemi-species of the group are more specialised than the Hetero species, the exceptions being U. ver craccae and U. f. eduardii. On the other hand all the Hetero-species are plurivorous except U. loti, U. ver. craccae and U. f. eduardii.

5. All the British heteroecious members of the group, belong to species for which races have been reported on the Continent, e.g. U. punctatus, U. pisi, U. striatus, U.laburni. The exception is U. loti for which no races has been reported.

6. All the heteroecious rusts can have E. cyparissias as an aecidial host ecept U. ver. craccae.

Discussion./

Discussion.

From the above, it appears that heteroecism in the group is confined to Central and S. Europe. The exception is U. pisi whose aecidial stage has been recorded both for S. Norway (Jørstad 1948) and S. England (Coombe 1953, Robertson, 1953). E. cyparissias is native in Central and S. Europe, and as has been pointed out above, it is the only aecidial host for all the heteroecious rusts under discussion except U. ver. craccae. Since heteroecious species are found outside this region, it is obvious that these species can overwinter in the uredo stage. In fact, U. striatus and U. punctatus are well distributed in other parts of the world e.g. N. America (Arthur 1934), where their possible aecidial hosts are absent. Jørstad (loc.cit.) suggests that in such cases where the rust exists outside its aecidial host distribution, the rusts have lost their ability to produce aecidia. Evidence in support of this has been presented earlier in this thesis in the discussion on U. punctatus.

In general, the members of the U. pisi group outside Central and S. Europe exist only in the uredo stage. For example, U. pisi on Lathyrus pratensis, U. loti on Lotus corniculatus, U. laburni on Caragana arborescens, etc. although mainly found in Eurasia, are also found where the aecidial host is absent. U. pisi on Pisum sativum, on the other hand, is/

is rarely found outside the distribution range of E. cyparissi and it is suggested that this is due to the fact that Pisum species are annuals, and consequently no green parts of the ho plant is available on which to overwinter the rust. When U. pisi on Pisum does occur outside this area it is usually sporadic and is probably due to wind borne spores from the South (Jørstad loc.cit.).

U. f. eduardii and U. ver. craccae, are similar in thi respect, in that they have never been found outside the regio in which the aecidial hosts are found. Yet the uredo host o those two rusts (Vicia cracca) is widely distributed in Europ

The decrease in the number of leguminous rusts in Britain and the specialised nature of the British species can be explained to a certain extent, by the limited number of possible hosts in this country. Nevertheless, certain of th British representatives of the heteroecious continental speci have more possible uredo hosts than actual uredo hosts in Britain, and this would suggest that these British species ha become very specialised.

Biological races have been reported only for heteroec- ious species, whereas the Hemi-species are usually highly specialised. c.f. U. pisi and U. l. latifolii. It is a fact also that no races have been reported for the heteroeciou species existing in the Hemi- form which occur outside the region/

region of distribution of the aecidial host. These facts suggest that hybridisation and crossing between species and races etc., can take place in the aecidial stage. (Craigie 1927 etc.) That this is possible is illustrated by Guyot's (1939) experiments where he inoculated various legumes with accidiospores on E. cyparissias, and obtained in one case infections upon Pisum sativum and Cytisus laburnum simultaneously, and in another case on P. sativum, P. arvense and Lathyrus aphoca. As Jørstad (loc.cit.) points out the inoculum must have been a mixed culture.

No doubt climatic conditions too have affected the distribution of these rusts to a certain extent. For example (Jørstad, loc.cit.) has shown that U. loti is found only in the lowest coastal areas of S. Norway, whereas the uredo host Lotus corniculatus is also found inland. Also U. vic.cracca and U. heimerlianus are both found on the warmer Continent but are absent from Britain although V. cracca a uredo host i well distributed in this country.

Conclusion.

From the available data on the ecological conditions and geographical distribution of these species, it is impossible to draw any definite conclusion to account for the distribution of the group. However it appears that heteroecism/

heteroecism in the group is mainly confined to Central and S. Europe and that outside this region, the heteroecious species adopt the Hemi- form of life cycle and become more specialised in their selection of hosts. This may be due to either or both of the following, (1) the absence of the aecidial host, (2) climatic conditions not favourable to the development of aecidia. It seems too, that the Hetero-forms outside the Central and S. European region have lost their ability to produce aecidia. It is suggested that the Continental Hemi- species have probably arisen from special-ised races of certain of the heteroecious species.

VI. Cytological data.

(a) Introduction.

In a cytological investigation of a group of organisms, the usual method is to compare the sizes, shapes, general morphology and the numbers of the chromosome complement of each member of the group. As already pointed out, such a method is not applicable to the U. pisi group of fungi because the nuclei of these organisms are only about 4μ in diameter. Other method of studying nuclei and their composition are quoted by Barber & Callan (1944), but these are equally difficult if not impossible to apply for the reason stated. Apart from optical techniques for investigating nuclear chemistry, the principal method has been that of gross chemical analysis. In this meth biochemical analysis is carried out in a known number of isolated nuclei, (Vendrely & Vendrely 1948,1949; Mirsky & Ris 1949; Davidson & McIndoe 1949; Pollister etal 1951; Thomson 1953 and others) and the amount of Desoxy-ribose nuclei acid (D.N.A.) per nucleus is computed. From the results of these workers, it has been shown that the D.N.A. content of somatic nuclei has a constant value for one species and that th diploid nucleus has twice as much D.N.A. as the haploid nucleus

Optical methods of investigation, in which the microscop is adapted as a light measuring instrument, have been used chiefly by the Caspersson school in Stockholm using ultra viole light (U.V.), and the Pollister school of Columbia University using/

using fixed Feulgen stained material in the visible spectrum. These cytophotometric methods measure the absorption of U.V. light by living muclei (Caspersson 1936, 1942, 1950 etc.), or t absorption of the mercury green line of the spectrum by Feulge stained nuclei. The measurements can be made either indirectl by taking photographs of the nuclei and determining the density of the nuclear images, or directly, by measuring the current ou put of a photo-cell placed at the eyepiece end of the miscrosco

By using such optical methods results have been obtained which agree well with those of chemical analysis (Leuchtenberge et al. 1951; Naora 1951; Basteels & Lison 1950; Ris & Mirsky 1949; Swift 1950; and others). Polyploidy has also been determined by these authors. The close agreement between thes results and those of chemical methods has served to validate th empirical methods of cytophotometry. The literature on this aspect has been critically reviewed by Pollister (1952), who states that "The conclusion is that in non-dividing tissue, the average amount of D.N.A. per chromosome complement is relativel constant ....."

It would appear then, that since the D.N.A. content for somatic muclei has a constant value for a particular species, this fact could be made use of in taxonomic studies. It woul only be of value, however, when different D.N.A. values were obtained for different species c.f. chromosome counts.

In/

In the rust fungi it is impossible to separate the mycelium-somatic tissue from the host tissue in order to provid a sufficient number of nuclei for gross chemical analysis. Even if spores were used it would be questionable if the spore coat could be ruptured to liberate the nuclei. The cytophotometric method of investigation lends itself well to the study o rust nuclei since ordinary paraffin sections stained by the Feulgen technique can be used. This method has been used in the present study.

The validity of the method depends on two factors. (1), the specificity of the Feulgen reaction to stain the D.N.A of the nucleus in situ, and (2), a constant relationship betwee the amount of D.N.A. present and the amount of regenerated basi fuchsin.

(1) The Feulgen reaction was deweloped as a specific stain for D.N.A. by Feulgen & Rosenbeck (1924), and Feulgen (1927), an has been used extensively by cytologists for staining cell nucl and chromosomes (Milowidow, 1938). The reaction involves the acid hydrolysis of the tissues by N/1 HCl at 60°C, for 4-40 minutes depending on the fixative used (e.g. de Tomasi 1936; Bauer 1932, 1933; Hillary 1939; di Stefano 1948a, 1948b; and others). It is specific for the desoxysugars of D.N.A. The exact nature of the reaction is not fully known but it is considered that the acid hydrolysis breaks the sugar linkages attached to the purine bases. This exposes the 2-desoxysugars which/

which remain attached to the phosphates in the furanose form. This form is immediately converted into the aldehyde form claimed by Stacey et al 1946; Chong Fu Li & Stacey 1949, Overend & Stacey 1949, to be ω-hydroxy laevulinic-aldehyde. I is this aldehyde which reacts with the Schiff reagent to produ an insoluble magenta coloured compound. (See also Serra 1943 Stowell 1946, 1952; Brachet 1946; di Stafano 1948a, 1948b; Davidson 1950; Barber & Price 1940, and others).

The above interpretation has been challenged by Semmens 1940; Choudhuri 1943; Carr 1945; and Stedman & Stedman 1943a,b, 1944, 1947,a,b, 1948; who claim that the dye compound produced in the reaction with the Schiff reagent is water soluble and is avidly absorbed by the non-histone protein, chromosomin, of the cell nucleus. They also claim that although the dye is produc only in the presence of D.N.A. it is proteins rather than D.N.A which are stained and that in any case the D.N.A. is destroyed by the hydrolysis procedure.

This view has been vigorously refuted by many workers, e.g. Callan 1943; Caspersson 1944; Barber & Callan 1944; Dobson 1946; Brachet 1946; 1947; Stowell 1946, 1948, 1952; and Ris & Mirsky 1949-50. These authors produced a large body of experimental data which could not be interpreted from the Stedmans' viewpoint. They also pointed out that the Stedman school had published no experimental details of their work.

The accepted view today is that the Feulgen reaction is specific/

specific for, and reveals the site of D.N.A. if carried out und carefully controlled conditions. This has been verified by recent work (Sibatani & Fukuda 1953).

(2) The Cytophotometric determination of the amount of D.N in tissues from Feulgen stained material, has been used increasingly in the past 15 years or so. Although the method has bee largely empirical in approach, the results of investigators in this field have been substantially corroborated (a) by chemical analysis (see above) (b) by cytophotometric analysis on living cells using the U.V. (Caspersson 1950, and (c) by other methods (Pollister 1952). The last author presents a substantial bod of evidence in his comprehensive review of the literature on th subject, and concludes that the amount of basic fuchsin regenerated in the Feulgen reaction must bear a constant relation ship to the amount of D.N.A. present in the nuclei of a conside able variety of tissues within one species.

(b) Cytophotometry.

(i) Theory and methods in cytophotometry.

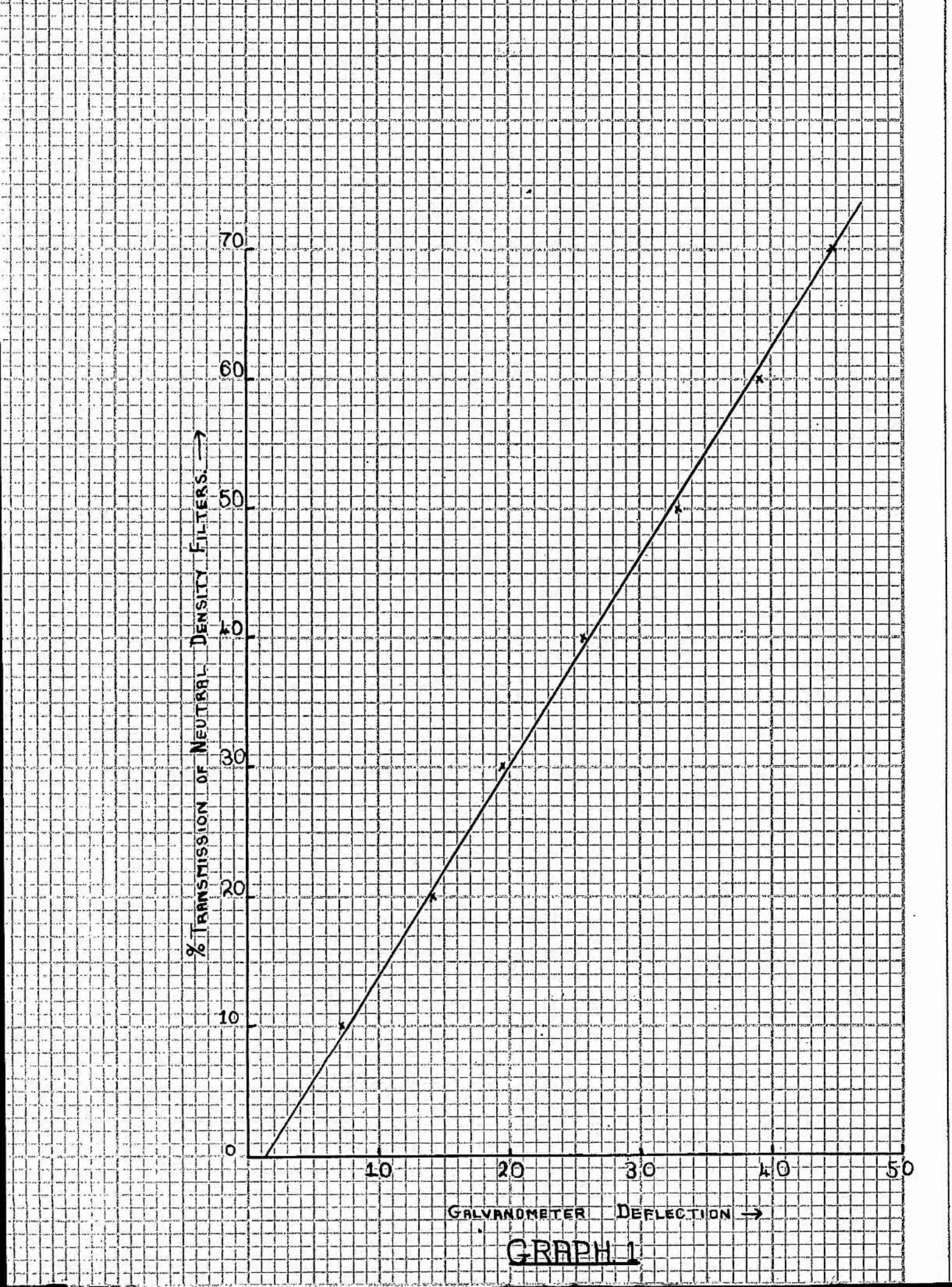
The literature on the theory and methods in cytophotomet has been reviewed by Caspersson (1950); Pollister et.al (1951) and Pollister 1952.

Most of the investigations in this field have been directed towards the elucidation of the chemical nature of the gene of which D.N.A. has been considered to be a component.

Photometric/

Photometric methods are based on the Beer-Lambert laws o absorption, which broadly speaking state, that the amount of light energy absorbed by a substance is a function of the numbe of molecules of absorbing substance in the light path. In practice, the amount of light absorbed in a solution, for instance, is expressed as a fraction or percentage of the light passing through the solvent alone. This fraction is known as the transmission T, and T= I/Io, where I = light absorbed b solute and solvent, and Io = light absorbed by solvent alone. In cytological preparations, I is the light absorbed by the stained nucleus, and Io is the light absorbed through a clear part of the slide, or more accurately, through an unstained nucleus.

By definition, the value of T depends on the number of absorbing molecules, i.e. the concentration 'c' and the thicknes 'd' of the region measured. Thus T∝kcd where k is a constant depending on the substance under test. T, however, does not vary directly with the number of absorbing molecules in the light path, but rather decreases logarithmically with a linear increase in the number of absorbing molecules i.e. if a given volume of a substance has a T value of 10, the same volume o the same substance of twice the concentration would have a T value of 1, and of three times the concentration, a T value of 0·1. In practice it is desirable to use a value which wil vary directly with the number of absorbing molecules, and this value/


70
60
50
40
30
20
10
0
% Transmission of Neutral Density Filters →
10
20
30
40
50
Galvanometer Deflection →

GRAPH 1

value is called the extinction, E.

Thus $E = k\,c\,d = \frac{1}{\log T}$.

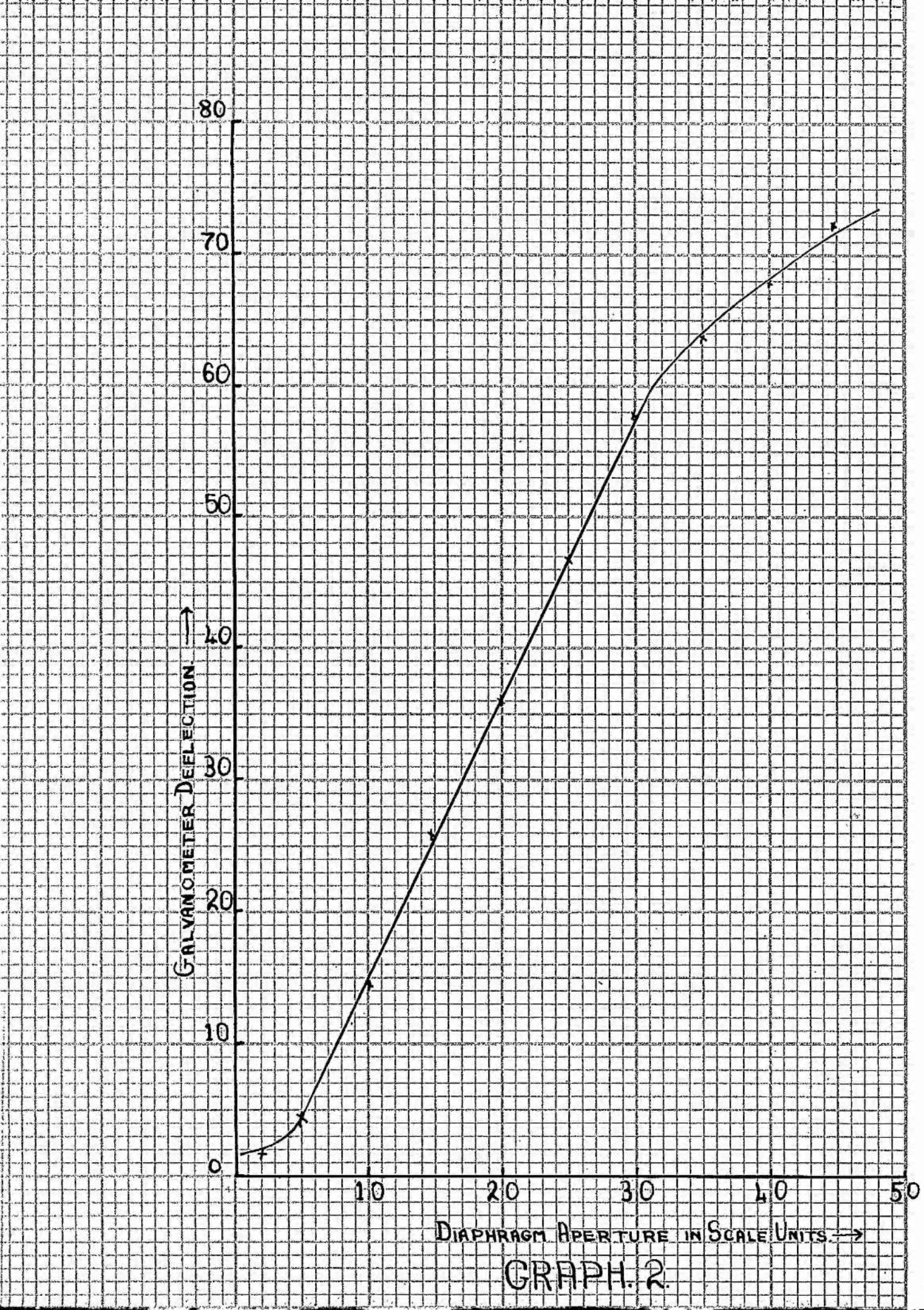
E = k c d, is only strictly true for a dye in solution, but it has been shown that Feulgen stained nuclei follow the closely relation/enough to permit the calculation of relative amounts of dye per nucleus in arbitrary units from extinction data (Ris & Mirsky 1949; Pollister & Swift 1950). The errors introduced vary but have been shown to be less than 5%

There are other sources of errors in cytophotometry and these are discussed below.

(ii) Response of electron multiplier tube (photocell).

The response, or electric current output of the cell, should be directly proportional to the intensity of incident illumination. In the present study, the linearity of the photocell response was tested by interposing neutral density filters in the light path and reading on the galvanometer the output of the cell for each filter. The results are given in Graph I. From this it is seen that the cell used had a linear response to varying light intensities.

Marshall et.al.(1948) have shown that the cathode of electron multiplier tubes is not uniformly photosensitive, but is practically uniform within an area of 5 mm. diameter round the exact centre of the cathode. Within this area and with a uniformly illuminated field, the galvanometer deflection is essentially


80
70
60
50
40
30
20
10
0
GALVANOMETER DEFLECTION →
10
20
30
40
50
DIAPHRAGM APERTURE IN SCALE UNITS →

GRAPH. 2.

essentially proportional to the area illuminated.

The electron multiplier cell used in the present study w tested for area response by taking a series of galvanometer readings at various apertures of the diaphragm placed in front of the photocell. The galvanometer readings were plotted against the aperture diameters and the resulting graph shows th results (Graph 2).

(iii) Purity of light source.

In the apparatus used in this study, monochromatic light was obtained by isolating the green line of the spectrum from a High Pressure Mercury Arc lamp by a special Wratten No. 77 filter. This filter transmitted 78% of the incident radiation at 550 m.u. The spectral transmission of this system is not, of course, pure, but according to Drabkin (1950), and Moses (1952), the small loss of spectral purity by using such broad band filters is justified in order to obtain the necessary intensity.

(iv) Scattering of light in the optical system.

Biological material is considered to be composed of colloidal particles, and these will scatter light whether they are stained or not. For example, if two structures, say a nucleus and its nucleolus have different refractive indices fro the surrounding medium, they will reflect and refract the light passing through the section. This scattering can affect the extinction/

extinction in two ways -

(1) If the light is scattered out of the measured beam due to t structure of the object, the E value will be too high (Barer 1950, Randall 1950, Scott 1952, Pollister 1952). From the theoretical point of view, this scattering can be considerably reduced if the tissue is mounted in a medium of refractive inde approaching that of the tissue (Ornstein 1952). Neutral Cana balsam meets this requirement and was used in the present study Nevertheless, the preparations made were tested to determine th magnitude of this structural light loss. The unhydrolysed controls or blanks for the Feulgen tests were used for this purpose (Pollister & Ris 1947; Swift 1950; Moses 1952; and others). These slides showed unstained nuclei and had been prepared and treated exactly as the stained test slides, except that they were not hydrolysed in acid. Measurements of light loss on these slides showed that there was no significant loss of light between the unstained nucleus and a clear part of the slide, i.e. the scattering of light due to the structure of the nucleus was insignificant.

(2) If light is scattered into the measured beam from the surrounding tissue, the E value will be too low. This is a more difficult source of error to determine than (1) above (Randall 1950). There are two factors in the illuminating system which are of prime importance and must be taken into consideration. These are the size of the field illuminated, and/

and the angle at which the light converges on the object. The first is controlled largely by the lamp diaphragm and the secon by the substage diaphragm.

Consequently, extinction readings were calculated for different apertures of these diaphragms on small regions of spherical, Feulgen stained nuclei. Those of the host were use for this purpose since they were larger than the fungal ones an were between 8 and 10μ in diameter. It would be expected from these readings that regions in the centre of the nucleus (the thickest part) would have higher E values than those taken from the marginal areas (the thinnest parts). This was only true, however, when the apertures were small. Consequently in the present study the lamp diaphragm was stopped down to 1.5mm. aperture so that the area illuminated was only a few diameters greater than the nucleus being measured. Similar results have been obtained by Swift 1950; Pollister & Ris 1947; and Moses 1952. This procedure eliminated much of the glare and scatter ing from the surrounding tissues.

Naora (1951,1952), has questioned the validity of data obtained by cytophotometric methods on the grounds that the are of the slide illuminated is a number of diameters greater than the actual nucleus under test. According to this author, wit apertures such as have just been described above, internal reflections are set up when a large area of the slide is illuminated and this affects the E value. This internal glare/

glare is referred to as the Schwarzchild-Villiger effect. It would appear that Naora has based his objections mainly in theoretical considerations, e.g. he discusses plane surfaces, when the surfaces in the biological system are usually spherica and has neglected to consider certain procedures used and described in practice for the elimination of glare. His objections have been satisfactorily met and explained by Ornste & Pollister (1952); Ornstein (1952); and Scott (1952).

In discussing the angle of the illuminating cone, Moses (1952); and Uber (1939), state that the condenser diaphragm should be stopped down to, from 0.3 to 0.6 times the N.A. of th objective. This has been confirmed by Swift (1950); Polliste (1952); Bryan (1952); Scott (1952); Casperson (1950), and in th present work. With the condenser diaphragm at full N.A. the scattering is considerable. On the other hand, if the diaphra aperture is very small, say 1 m.m. then the increased diffracti causes the non-specific light loss to be large. In all the measurements carried out in the present study the condenser diaphragm was stopped down to 4 m.m. diameter and the condenser was focussed on the light source.

If the object being measured has a flat upper and lower surface, e.g. a slice of cytoplasm, then a cone of light from t condenser will obviously pass through more absorbing material than would a parallel beam, i.e. the optical path is greater tha the actual thickness of the object. Caspersson (1936) however has shown that in the case of a sphere, the mean lengths of the optical/

optical paths for converging and parallel light are very nearly identical.

The rust nuclei measured in the present study were all spherical or nearly so Plate VII (figs.35,36) and the mean optical path was calculated by converting the volume of the sphere to that of a cylinder of the same cross-sectional area a solving for the height 'h' of the cylinder (Swift 1950; Pollist 1952; Moses 1952; and others).

Vol. of cylinder = Vol. of sphere.

i.e. $\pi r^2 h = \frac{4}{3} \pi r^3$

$\therefore \quad h = \frac{4}{3} r$

The total amount of absorbing substance, A, in a cylinder can then be calculated:-

A = vol. x concentration

$= \pi r^2 h \times \frac{E}{k d} \qquad \because E = k c d.$

$= \pi r^2 \times \frac{E}{k} \qquad \because d = h$

Since k is a constant for the substance under investigation:

$A = \pi r^2 E$ i.e. the cross-sectional area x the extinct:

Stowell (1946), Serra (1943), Swift (1950), and others, state that in cytophotometric methods all extraneous light, e.g. daylight and room lights, must be eliminated, but in the present study it was found that daylight or the room light had not the slightest/

slightest effect on the readings. This is not surprising considering the very small apertures used in the system.

(v) Distributional Errors.

As already shown, the total mount of absorbing molecules in arbitrary units is calculated by multiplying the extinction E by the cross sectional area πr^2. Theoretically the number of molecules can be determined in this way only if they are randomly distributed and if every point within the measured image has the same intensity. In practice the image of a fixed stained nucleus is not uniform in intensity, both because of its shape and because the nuclear material is clumped in irregular masses. When the image of such a nucleus falls on the cathode of the photo-cell, the current response can be considered proportional to the sum of the various incident intensities. In this case I/Io will give an average transmission Ta. From this Ta value an average extinction Ea can be computed which will be proportional to the number of absorbing molecules within the nucleus. For most cytological material, this assumption is not strictly correct (Fano, 1947). It can be shown, that while for every point in the image there can be found a T. value and a corresponding E value, Ea $\propto$ Ta only when all intensities in the image are the same (Scott 1952; Moses 1952). This is because the relationship between E and T is a complex one involving a logarithmic function. For any point 'i' within the image

$$\frac{1}{\log Ti} = Ei.$$

But/

But in considering a large heterogeneous image

$$\frac{1}{\log T} \neq E.$$

The Pollister school has shown that nuclei after fixing in formalin appear to be homogeneous and this condition is very desirable in cytophotometric investigations. 20% and 30% Formalin was used as a fixative in the present study, but it was found that the enlarged image of the nucleus was indistinct at the margins which rendered it difficult to measure the diameter with any accuracy. Consequently, Carnoy's 1:3 Acetic-alcohol was used. Because the nuclei were small, clumping was not evident and the staining appeared uniform (Plate VII figs.35,36

The Caspersson school in Stockholm has probably contributed more than any other group to the study of the errors inherent in cytophotometric methods (Caspersson 1950 etc., Moses 1952). The two main points that Caspersson has dealt with are the shape of the image compared with the object, and the distribution of the light energy in the image and the object. The conclusion reached by this school is that in order to obtain a satisfactory image, the objective lens must have an aperture large enough to give full resolution of the object to be measured, in the particular wavelength of light used. Such an object should not be less than three times the wavelength of light used. Further all light leaving the structure being measured, must participate in forming the image, i.e. must enter the objective lens, otherwise/

otherwise the intensity distribution in the image will not correspond to that in the object. These conditions were fulfilled in the present study by using a condenser of N.A. 1.3, and an objective of N.A. 1.28. The sizes of the nuclei were well above three times the wavelength of the green mercury line of light.

Other errors due to such factors as dichroism, anomalous dispersion, high concentration of the absorbing layers and effect of neighbouring molecules, have been discussed by various workers, e.g. Caspersson 1940, Moses 1952, Scott 1952, Swift 1950, Pollister & Swift 1950, Commoner 1949. It appears, however, that these factors have not so far been shown to be of great importance in the type of cell studies under discussion (Glick et al. 1951, Scott & Sinsheimer 1950; Moses 1952; Swift (1950).

There can be no doubt that all the errors discussed above are very complex and are not properly understood at present. The most promising way of checking the validity of cytophotometric results, as Pollister (1952) has emphasised, is to measure homogeneous objects as far as possible and to compare the results with direct biochemical analysis. This appears the more logical approach than to attempt,as has been suggested, by implication at least, (Commoner 1949; Commoner & Lipkin 1949; Glick et al. 1951), to assess and correct for all errors by computation from other physical data on cells, data which are hardly/

hardly obtainable at present with the necessary accuracy (Pollister, loc.cit.)

In the present work it has been impossible to carry out direct biochemical analysis, and the validity of the results obtained is based on the allowance made for the above errors as far as possible, and the use of an apparatus similar in construction to those described in the literature where results have been checked by chemical analysis and other methods.

(c) Cytophotometric investigation of rust fungi.

(i) Description of apparatus.

The apparatus used is shown in Plates VII,VIII,figs.37,38,39. Essentially it consists of a light source producing a monochromatic parallel beam,which passes through a compound microscope and is projected on to the cathode of a photocell, whose current output is measured by a sensitive galvanometer. The entire optical system is mounted on an optical bench for rigidity and ease of adjustment. The light source has already been described. I, is the stabilising choke for the lamp H, which is a 500 watt type, and F is the Wratten No. 77 filter. This filter selects out the 546 mμ wavelength at which the regenerated Feulgen dye has its maximum absorption. A collumating lens and the lamp diaphragm are mounted at G, and can be centred by three centring screws. The diaphragm has a scale which reads the apertures in millimetres. The substage apparatus consists of a Watson's Holoscopic oil immersion condenser of

N./

N.A. 1.3, and a diaphragm. The latter also has a scale, readi the apertures in millimetres. The objective is a Watson's 100 Parachromatic oil immersion 2 mm. of N.A. 1.28, and the ocular a 10 x compensating eyepiece. The microscope is mounted horizontally on an adjustable platform E. The bellows are tho used in ordinary microphotography and have a shutter diaphragm inserted at the narrow end. When the apparatus is used for microphotography, the carrier at D receives the photographic plate holder which is interchangeable with a ground glass plate for focussing. This ground glass plate (Plate VII, fig.38) was also used in the present study for rough focussing and centring, and to search the field to select individual nuclei for measurement. It was interchangeable with a tube panel D (Plate VIII, fig. 39) in which a tube, fitted with an internal iris diaphragm, was mounted in the exact centre of the optical axis. A pointer which was attached to the diaphragm, moved ov a scale and so enabled the diameter of the aperture, which circumscribed the nuclear image, to be measured. The tube cou receive either an ordinary eyepiece for exact focussing and centring, or a photocell C (Plate fig.37). The latter w an R.C.A. 931A electron multiplier tube in a light tight case. It has 9 dynodes and operates at 100 volts per dynode stage on voltage supply of 900 volts. The present tube was operated at a voltage of 840-60 volts supplied by the voltage doubler and rectifier unit B. Text fig. 2 gives the circuit diagram of this/

Text, Fig. 2.

Text Fig. 2. Circuit diagram of Voltage Doubler Unit. (Plate VII, Fig. 37).

this unit. The current output of the photocell was measured with a Marconi galvanometer A, with a full scale deflection of 10μA in the most sensitive position.

(ii) Operating technique.

Before measurements were taken the lamp was allowed to run for 20 mins. to attain its maximum brilliance. Meanwhil the optical system was aligned using normal microscopic practi and employing Köhler illumination. After 20 mins. the shutte diaphragm of the bellows was opened and the image of the secti on the ground glass plate was searched for suitable nuclei (fi 38).. The ground glass plate was then replaced by the tube panel D, a 10x ocular was inserted in the tube, and the tube diaphragm was opened to maximum aperture. The nucleus to be measured was then carefully centred and focussed in the field, and the disphragm was closed to circumscribe the nucleus. Th substage diaphragm was then stopped down to 4 m.m. and the lam diaphragm to 1.5 m.m. aperture.. A clear part of the slide was then brought into the field, after which the ocular was replaced by the photocell. The substage and lamp diaphragms were then slightly adjusted until the galvanometer read approximately 60. These diaphragms were left in these positi throughout the whole series of measurements. The photocell i removed and the tube panel D is replaced by the ground glass plate. The same nucleus is then brought into position when t

plate/

plate is again replaced by the tube panel D. The nucleus is again centered and focussed using the panel tube ocular, and is circumscribed by the tube diaphragm. The shutter of the bello is then closed and the ocular is replaced by the photocell. T shutter is then opened and the final focussing is done by sligh moving the fine adjustment. The nucleus is in focus on the cathode of the photocell when the galvanometer indicates a decrease in reading. The shutter is then closed for 30 secs. to rest the photocell, and then opened when the reading `In' through the nucleus is taken. The shutter is then closed. This last part of the procedure was repeated 3 times and the mean taken. Variation was only in the region of 1 to 2 divisions on the galvanometer scale. The photocell was replaced by the tube ocular and a clear part of the slide was brought into the field. The ocular was then replaced by the photocell. All diaphragms were kept at the same aperture as set in the In readings. The reading `Io' through the clear part of the slide was then taken as for `In.' The scale on the tube panel D (fig. 39) was read giving the radius of the nucl The procedure just given provides all the data necessary for th calculation of the amount of D.N. A. per nucleus in arbitrary units. For each nucleus measured in the present study, such procedure was repeated three times. When the photocell was n in use, it was kept in the dark by covering the opening in the light tight case by a tinfoil shield. Before and after all t readings/

readings for each nucleus, the lamp was tested to determine if it was retaining its maximum brilliance. It was found that after about 1 hr. 20 mins. operation, the brilliance gradually fell off. Consequently after each hour of operation the lamp was switched off and allowed to cool for ½hr. before being used again.

(iii) Preparation of material.

Only the rusts U. punctatus, U. loti and U.pisi. were used in this experiment as they were the only living specimens available at the time the experiment was carried out. They were collected on 19/9/53 and fixed immediately in freshly prepared Carnoy's 1:3 Acetic alcohol. After fixing for 15 hours, the material was washed in 95% alcohol for 3 hours, and subsequently dehydrated by the tertiary butyl alcohol method according to Johansen (1948). After dehydration was completed the material was embedded in B.D.H. paraffin wax with ceresin added of C.P. 56 °C. Sections were cut at 6µ thickness and fixed to slides by Haupt's adhesive. 3 rows of sections were put on each slide, one of each species, to ensure uniformity of treatment. De-waxing was carried out in Xylene, and the slides were then run down an alcohol series and finally washed in running tap water for 4 hours, then rinsed in distilled water. The slides were then ready for staining.

They were placed in cold 1.01 N, HCl for 1 min. and then transferred to the hydrolysing vessel containing 1.01N, HCl, and hydrolysed/

Table 36.

No.	In	Io	r	$K\pi r^2$	Mean $K\pi r^2$
1	15	50	8	105.1	
	20	55	9	111.8	106.2
	19	54	8.5	102.9	
2	28	61	10	106.1	
	34	66	11	109.4	110.7
	29	63	10.5	116.8	
3	20	55	9	111.8	
	19	55	9	117.7	114.4
	23	58	9.5	113.9	
4	40	71	12	112.7	
	40	70	12	109.8	112.4
	36	08	11.5	114.8	
5	30	63	10.5	111.6	
	34	65	11	106.9	111.5
	29	63	10.5	116.2	
6	27	61	10	111.1	
	28	62	10	108.4	108.5
	28	61	10	106.1	
7	40	70	12	109.8	
	37	69	12	112.4	111.7
	42	71	12	103.1	
8	24	59	9.5	110.8	
	29	63	10.5	116.6	112.8
	27	61	10	111.1	
9	27	61	10	111.1	
	28	63	10.5	121.9	113.8
	28	62	10	108.4	
10	34	65	11	106.9	
	28	63	10.5	121.9	111.9
	34	65	11	106.9	
11	24	59	9.5	110.8	
	21	55	9	106.4	109.4
	27	61	10	111.1	
12	28	62	10	108.4	
	26	61	10	116.2	111.9
	27	61	10	111.2	
13	29	63	10.5	116.8	
	34	65	11	106.9	111.7
	30	63	10.5	111.6	
14	36	68	11.5	114.8	
	34	66	11	109.4	110.3
	34	65	11	106.9	

Table.36.Showing absorption data for U.punctatus. Explanation in text.

Table 37.

No.	In	Io	r	E πr^2	Mean E πr^2
1	27	63	10.5	127.5	
	31	66	11	127.5	126.4
	31	67	11	127.5	
2	21	58	9.5	125.0	
	17	54	9	127.6	126.6
	21	59	9.5	127.2	
3.	17	55	9	129.7	
	21	58	9.5	125.0	127.9
	21	60	9.5	129.2	
4.	25	62	10	123.9	
	20	59	9.5	133.1	128
	24	61	10	127.2	
5.	27	63	10.5	127.5	
	25	62	10	123.9	125.2
	24	60	10	124.9	
6.	25	61	10	121.6	
	27	64	10.5	131.0	126.6
	24	61	10	127.2	
7.	34	68	11.5	125.0	
	31	66	11	124.7	125.6
	31	67	11	127.2	
8	21	58	9.5	125.0	
	17	55	9	129.7	129.2
	20	59	9.5		
9	17	56	9	131.7	
	20	58	9.5	120.8	125.4
	25	62	10	123.9	
10	21	58	9	125.0	
	24	60	10	124.9	125.8
	27	63	10.5	127.5	
11	27	64	10.5	129.8	
	24	61	10	127.2	126.2
	25	61	10	121.6	
12	25	62	10	123.9	
	20	58	9.5	131.0	126.6
	24	60	10	124.9	
13	27	63	10.5	127.5	
	31	66	11	124.7	127.2
	31	68	11	129.6	
14.	21	59	9.5	127.2	
	22	59	9.5	121.4	123.4
	25	61	10	121.6	
15	24	61	10	127.2	
	27	64	10.5	131.0	130.2
	30	67	11	132.2	

Table 37. Showing absorption data for U.loti. Explanation in text.

Table 38.

No.	In	Io	r	E r	Mean E r	No.	In	Io.	r	E r	Mean E r.
1.	38	66	11	90.2		9	24	56	9	93.6	
	35	63	10.5	88.5	90.5		26	55	9	81.3	88.8
	34	63	10.5	92.7			24	55	9	91.6	
2.	32	61	10	87.9		10	18	50	8	89.2	
	31	61	10	92.2	90.2		14	48	7.5	94.5	90.1
	32	62	10	90.2			22	53	8.5	86.6	
3.	18	50	8	89.2		11	21	53	8.5	91.2	
	21	53	8.5	91.2	87.2		29	59	9.5	87.4	90.7
	26	55	9	81.2			24	56	9	93.6	
4.	14	47	7.5	92.9		12	18	50	8	89.2	
	17	50	8	94.2	94.3		22	53	8.5	86.6	87.6
	17	51	8	95.9			25	55	9	87.1	
5.	25	56	9	89.1		13	17	50	8	94.2	
	29	59	9.5	87.4	90.0		21	54	8.5	93.0	92.1
	24	56	9	93.6			18	50	8	89.2	
6.	31	60	10	90.0		14	25	56	9	89.1	
	30	60	10	94.5	93.4		31	60	10	90.0	90.9
	37	66	11	95.5			24	56	9	93.6	
7.	34	64	10.5	95.1		15	31	61	10	92.3	
	31	62	10	94.5	93.8		29	59	9.5	87.4	89.9
	38	59	9.5	91.7			31	60	10	90.0	
8.	17	50	8	94.2		16	44	70	12	91.2	
	21	53	8.5	91.2	91.5		37	66	11	95.4	91.7
	18	50	8	89.2			38	65	11	88.6	

Table 38. Showing the absorption data for U.pisi. Explanation in text.

hydrolysed for 12 mins. at 60°C. Following this they were washed in cold acid and then transferred to the Feulgen reagent prepared according to de Tomasi (1936) and stained for 30 mins. Subsequent bleaching was carried out with SO_2 water (Johansen 1948). The slides were then run up the alcohol series and finally dehydrated in absolute ethyl alcohol, then mounted in neutral Canada Balsam dissolved in Xylene.

In the above procedure, the slides were treated identicall as far as possible.

The unhydrolysed controls were treated exactly as the stained test slides, except that during the hydrolysis period they were kept in distilled water. None of these slides had taken up any stain and there was no trace of the plasmal reactio

(iv) Results and conclusions.

The results obtained by measuring the light absorption of Feulgen stained nuclei of U.Punctatus, U.loti and U.pisi are given in tables 36, 37, and 38, respectively. In these tables, In is the reading through the nucleus, Io the reading through a clear part of the slide, r is the radius of the nucleus in arbitrary units (actually 4 divisions on the tube pane D scale = 1μ) and $E \pi r^2$ is the computation of the relative amount of D.N.A. in arbitrary units. Each nucleus was measured 3 times as already explained and the means of these readings are given in the sixth column.

The data given in the sixth columns of Tables 36, 37, & 3 were treated as follows:- The means were taken and the standard deviations calculated. Using the 'student t test' the means of the species were tested to determine if there was any significant difference between them. The 'significance' wa calculated at a 5% level, and values above 2.05 were significantly different. The results are given below:-

Mean of U.punctatus c.f. mean of U.loti: 21.7
" " " " " " of U.pisi: 27.0
" " U.loti " " " " " : 153.8

From these results it was concluded that there was a significant difference in the relative amounts of D.N.A. per nucleus between the species U.punctatus, U.loti and U.pisi.

This would suggest that these organisms are in fact different species. In order to lend support to this suggestion it was decided to determine the relative D.N.A. content per nucleus of two varieties of Puccinia graminis Pers. Through the courtesy of Prof. T. Johnson of the Dominion Rust Research Laboratory, Winnipeg, Canada, the two varieties, P.graminis tritici on wheat and P.g. avenae on oats were obtained. Prof. Johnson had fixed fresh living material in Carnoy's 1:3 Acetic alcohol for 15 hours, and then stored it in 70% alcohol in sealed vials for transit to St. Andrews. The material was in this fluid for 7 days. At St. Andrews the material was embedded/

Table 39.

No.	In	Io	r.	$E\pi r^2$	Mean $E\pi r^2$.	No.	In	Io.	r.	$E\pi r^2$	Mean $E\pi r^2$.
1	36	47	7.5	20.5		9	38	51	8	25.7	
	35	47	7.5	22.6	22.3		34	47	7.5	24.8	23.7
	38	50	8	23.9			36	47	7.5	20.5	
2	32	44	7	21.8		10	16	32	5	23.6	
	29	41	6.5	19.9	21.2		17	32	5	21.6	22.6
	31	43	7	22.4			17	33	5	22.6	
3	31	44	7	23.4		11	29	41	6.5	19.9	
	31	43	7	21.9	23.2		25	39	6	21.9	21.0
	35	48	7.5	24.2			24	37	6	21.3	
4	24	38	6	22.6		12	32	44	7	21.3	
	22	35	5.5	19.2	20.9		31	43	7	22.4	22.4
	21	35	5.5	21.1			31	44	7	23.4	
5	17	33	5	22.6		13	29	42	6.5	22.7	
	16	32	5	23.6	22.4		28	41	6.5	22.0	22.2
	21	35	5.5	21.1			25	39	6	21.9	
6	24	38	6	22.6		14	16	32	5	23.6	
	25	39	6	21.8	21.6		21	34	5.5	20.1	21.7
	25	38	6	20.6			24	37	6	21.3	
7	31	44	7	23.4		15	32	44	7	21.3	
	29	42	6.5	22.7	22.7		35	47	7.5	22.6	22.6
	38	41	6.5	22.0			38	50	8	23.9	
8	32	43	7	19.7							
	29	41	6.5	20.6	21.0						
	31	44	7	23.4							

Table 39. Showing the absorption data for P.graminis tritici on wheat. Explanation in text.

Table 40.

No.	In.	Io.	r.	E r	Mean E r	No	In	Io	r	E r.	Mean E r
1	38	50	8	23.9		9	32	43	7	19.8	
	39	51	8	23.4	23.3		29	42	6.5	22.7	22.0
	35	47	7.5	22.6			31	44	7	23.4	
2	36	47	7.5	20.5		10	38	51	8	25.7	
	32	44	7	21.3	21.4		35	47	7.5	24.5	24.0
	31	43	7	22.4			38	50	8	23.9	
3	25	38	6	20.6		11	17	33	5	22.6	
	25	39	6	21.8	21.2		25	37	6.0	19.2	21.0
	24	37	6	21.3			21	35	5.5	21.1	
4	25	38	6	20.6		12	24	38	6	22.6	
	29	41	6.5	19.9	21.0		21	34	5.5	20.1	21.3
	24	38	6	22.6			24	37	6	21.3	
5	16	32	5	23.6		13	31	44	7	23.4	
	17	32	5	21.6	21.4		32	44	7	21.3	22.2
	22	35	5.5	19.2			28	41	6.5	22.0	
6	31	44	7	23.4		14	37	50	8	26.3	
	31	43	7	21.9	22.2		36	47	7.5	20.5	23.4
	32	44	7	21.3			39	51	8	23.4	
7	24	38	6	22.6		15	25	38	6	20.6	
	28	41	6.5	22.0	22.4		25	39	6	21.8	21.6
	29	42	6.5	22.6			24	38	6	22.6	
8	38	50	8	23.9							
	34	47	7.5	24.8	24.3						
	35	48	2.5	24.1							

Table 40 Showing the absorption data for P.graminis avenae on oats.
Explanation in text.

embedded, sectioned and stained, exactly as for the U. pisi group material. The absorption data are given in Tables 39 and 40.

The data in the sixth columns of Tables 39 and 40 were analysed in the same manner as the U. pisi group data. Significance was calculated at 5% level and the results are given below.

P.g. avenae cf. P.g. tritici : 0.17.

Therefore there is no significant difference between the amounts of D.N.A. per nucleus in these varieties. These varieties are identical morphologically and can only be separated by host reaction. It is unlikely, therefore, that this difference in host reaction is due to a difference in chromosome numbers and this is supported by their having the same D.N.A. content per nucleus.

In the U. pisi group, on the other hand, the members can be separated morphologically and to a certain extent by host reaction. Those members of the group which have been examined cytologically have been shown to have different amounts of D.N.A. per nucleus, and it is concluded, therefore, that they are distinct species. In view of the difference in D.N.A. content per nucleus, it is suggested that those species examined have different chromosome numbers.

Conclusions/

VII. Conclusions.

The members of the U. pisi group have been examined from three standpoints, viz:- the morphological, the host specialisation, and the D.N.A. content per somatic nucleus.

The data which have been presented for the first and the third of these, have indicated that the members of the group are distinct species. The separation of the members is more difficult when their host specialisation is considered for as has been shown, more than one species of rust may infect the same species of host plant. Nevertheless, when this does occur, it has always been found possible to separate the species by spore measurements.

It is concluded, therefore, that although the members of the group probably have a common ancestor, and resemble each other both morphologically and in their host reaction there is sufficient difference between them to justify each being given specific rank.

VIII. Summary.

1. The difficulties in separating the individual members of the Uromyces pisi group are outlined.

2. A review of the literature on rust fungi is given in which it is brought out that the major criteria for delimiting the species are (i) dimensions, wall thickness, number of germ pores and their distribution, for uredospores, (ii) dimensions, thickness of wall and pore cap, and wall sculpturing of the teleutospores, (iii) host specialisation, and (iv) length of life cycle.

3. 76 herbarium specimens along with the locally occurring members of the U. pisi group were examined from these stand-points, and the relative desoxy-ribose nucleic acid (D.N.A.) content per nucleus of U. pisi, U. punctatus and U. loti was determined.

4. It was found that the species of the group could be separated by statistically analysing the spore measurement Only U. jordianus could be separated by the number of germ pores of its uredospores, and both spore types of all the species had approximately the same wall thickness. It is shown that the sculpturing of the teleutospore wall varies within each species and shows a gradation from a weakly punctate, to a definitely punctate-striate condition.

5./

5. A review of the literature on host specialisation and life cycle is given for each species, and a host list and synonym list has been compiled for each. From this review it is pointed out that, (i) it is difficult to separate certain of the species of the U. pisi group by host reaction and (ii) biological races exist in some of the heteroecious species.

6. Inoculation experiments were carried out with the locally occurring U. punctatus on Astragalus danicus, and it was found that the rust could infect 9 other species of Astragalus and one of Medicago. Attempts to germinate the teleutospores by various chemical stimulants failed with the exception of one teleutospore. Observation and experiment showed that U. punctatus could overwinter by means of uredospores. Inoculation experiments were carried out on the aecidial host, but no infection ensued after four years. It is concluded that U. punctatus at St. Andrews has lost the ability to infect the aecidial host and that this is due to, (i) the absence of the aecidia host over a period of years leading to adaptation to the uredo host, and (ii) various climatic factors.

7. Inoculation experiments carried out with the local specimens of U. pisi on Lathyrus pratensis and U. loti on Lotus corniculatus, showed that these rusts were narrowly specialised on their respective hosts.

8./

8. Other closely related rusts which exist in the Hemi- form and are highly specialised with regard to host selection, are described and it is suggested that these rusts have probably arisen from specialised races of the heteroecious members of the group.

9. The ecology and geographical distribution of the group is discussed. The number of leguminous rusts in Britain is less than the number on the Continent. With the exception of U. pisi, heteroecism in the group is confined to Central and S. Europe. Outside this region the heteroecious species adopt the Hemi- form and can overwinter in the uredo stage. In this condition they become more specialised. The number of possible British hosts is greater than the actual number parasitised, except U. loti and U. laburni where the actual numbers are the same as the possible numbers of hosts.

In considering these points it is suggested that the absence of the aecidial hosts from the region outside Central and S. Europe, has led to the species adopting the Hemi- form and becoming more specialised. Also in this region the climate is not favourable to the development of the aecidium.

10. It is suggested on this evidence, that the members of the group probably have a common ancestor.

11./

11. The relative D.N.A. content per somatic nucleus was determined in arbitrary units by measuring the absorption of the 550 m.u. wavelength of the Mercury band of the spectrum, by Feulgen stained nuclei. The results showed that U. punctatus, U. pisi, and U. loti, which were the only species examined, had significantly different relative amounts of D.N.A. per nucleus. It is concluded on this evidence that these rusts are separate species and that the difference in amount of D.N.A. per nucleus is due to a difference in chromosome numbers.

12. The relative D.N.A. content per somatic nucleus was determined in the same way for the morphologically identical varieties, Puccinia graminis tritici and P.g. avenae. There was no significant difference in the relative amounts of D.N.A. per nucleus between these varieties. It is concluded that the host specialisation exhibited by these rusts is due to gene rearrangement. Those results lend support to the conclusion in 11. above.

13. From all the evidence presented, it is concluded that the members of the U. pisi group examined and described in this study are separate species.

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Plate I. Figs. 1-6. *U. punctatus*: Showing sculpturing on teleutospore walls. Phase contrast x 1,000. Enlarged 3 x.

Plate II. Figs. 7-12. *U. striatus*: Showing sculpturing on teleutospore walls. Phase contrast x 1,000. Enlarged 3 x.

Plate III. Figs. 13-18. Figs. 13-17: U. loti: Showing sculpturing on teleutospore walls. Phase contrast x 1,000. Enlarged 3 x.

Fig. 18: U. laburni: Showing sculpturing on teleutospore walls. Phase contrast x 1,000. Enlarged 3 x.

Plate IV. Figs. 19-24. U. pisi: Showing sculpturing on teleutospore walls. Phase contrast x 1,000. Enlarged 3 x.

Plate V. Figs. 25–30.

Figs. 25–26: U. pisi: Showing sculpturing on teleutospore walls. Phase contrast x 1,000. Enlarged 3 x.

Figs. 27–29: U. jordianus: Showing sculpturing on teleutospore walls. Phase contrast x 1,000. Enlarged 3 x.

Fig. 30: U. fischeri-eduardii: Showing sculpturing on teleutospore walls. Phase contrast x 1,000. Enlarged 3 x.

Plate VI. Figs. 31-36.

Fig. 31: *U. fischeri eduardii*: Showing sculpturing on teleutospore walls. Phase contrast x 1,000. Enlarged 3 x.

Figs. 32-33: Dividing somatic nuclei of *U. loti*. Fixed in Carnoy 3:1 Acetic-alcohol and stained by the Feulgen technique. x 1,000. Enlarged 3 x.

Fig. 34: Showing incubation chamber.

Figs. 35-36: Paired nuclei of *U. punctatus* selected for D.N.A. measurements. Fixed in Carnoy 3:1 Acetic-alcohol and stained by the Feulgen technique. x 1,000. Enlarged 3 x.

Plate VII. Figs. 37-38. Showing apparatus used in D.N.A. determination of Feulgen stained nuclei. Explanation in text P.137.

Plate VIII. Fig. 39. Showing apparatus used in D.N.A. determination of Feulgen stained nuclei. Explanation in text p.137.